Author:
Noll, Madeleine E

Title:
The Control of Stomoxys calcitrans (Stable Flies) with Essential Oils

The Control of *Stomoxys calcitrans* (Stable Flies) with Essential Oils

Madeleine Noll

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of MSc (Res) in the Faculty of Life Sciences, School of Biological Sciences.

10th August 2020

Word count: 14,764

Abstract

Stable flies are important hematophagous ectoparasites due to their broad range of mammalian hosts and world-wide distribution. As a result of their interrupted feeding behaviour, stable fly biting can result in a suite of direct and indirect adverse effects for their hosts. When densities are high, stable fly control is important particularly in dairy and beef cattle systems, on economic and welfare grounds. However, recently, the negative environmental and health consequences associated with exposure to conventional synthetic insecticides have become evident as well as the increasing development of resistance. Consequently, there is a need for an environmentally substantiable and effective alternative mechanism for stable fly control to be identified. The work set out in this thesis aimed to evaluate the efficacy of essential oils as insecticides and repellents for stable flies.

A semi-quantitative literature analysis of essential oils against biting flies suggested that lavender and tea tree oils were likely to be effective and hence these oils were chosen for investigation against stable flies. Using laboratory bioassays using laboratory bioassays in the stable fly, Stomoxys calcitrans, 5% (v/v) lavender and tea tree essential oils with ethanol excipient, caused 100% mortality for 4 and 6 h after exposure, respectively. In repellency bioassays, 5% (v/v) lavender and tea tree oils were able to deter 83.3% and 90% of stable flies from crossing an impregnated filter paper funnel for 1 h, respectively. The repellency of these essential oils was greater than that of a commercial repellent (20% DEET) which repelled 63.3% of flies for 1 h. The effectiveness of these oils *in vitro,* suggests that future work should focus on examining their potential *in vivo.* If effective in the field*,* these oils pose as viable alternatives to conventional synthetic treatments used in high value animal husbandry, particularly if issues associated with their cost and limited residual activity can be overcome.

Dedication and acknowledgements

There are several people I would like to acknowledge for their assistance and support during the research and writing of this project. Firstly, I would like to express my gratitude to my supervisor Professor Richard Wall for his encouragement, support and invaluable guidance throughout this project. I would also like to thank Dr Bryony Sands and Oliver Souter for sharing their knowledge with me which helped tremendously. I would also like to extend my thanks to my fellow MSc students, Katie Bryer, Amber-Rose Cooper and many others for their continual encouragement, support and for making this such a fun year.

Author’s Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

Signed: Madeleine Noll Date: 10th August 2020

Table of Contents

List of Figures

Chapter 1: Introduction: *Stomoxys calcitrans,* their veterinary importance and control mechanisms

Chapter 2: Efficacy of the essential oils against *Stomoxys calcitrans* in *in vitro* experiments

List of Tables

Chapter 1: Introduction: *Stomoxys calcitrans,* their veterinary importance and control mechanisms

Chapter 1: Insecticidal and repellent effects of lavender, *Lavandula angustifolia,* and tea tree, *Melaleuca alternifolia,* essential oils against stable flies.

Chapter 1

Stomoxys calcitrans - veterinary importance and control

1.1 *Stomoxys calcitrans*

The genus *Stomoxys* (Diptera: Muscidae) is comprised of eighteen species, including the stable fly (*Stomoxys calcitrans*) (Zumpt, 1973; Dsouli *et al.,* 2011). *Stomoxys*, meaning 'sharp mouth', are unique within the Muscidae as the adults of both sexes are obligate hematophagous ectoparasites of mammals (Foil and Hogsette, 1994). Unlike most stomoxine species, which are found exclusively in the tropics, stable flies are cosmopolitan pests with a world-wide distribution. This, in combination with their extensive range of mammalian hosts makes them of veterinary importance (Zumpt, 1973; Foil and Hogsette, 1994).

Stable flies are also referred to as 'biting house flies' due to their resemblance to common house fly, *Musca domestica*, in size and shape, with female adults being approximately 7mm in length and males being slightly smaller (Foil and Hogsette, 1994; Masmeatathip *et al.,* 2006). However, they are distinguishable due to their colouration; they are lighter and greyer in colour with four longitudinal darkened stripes on their thorax and black checkering on their abdomen (Fig. 1) (Masmeatathip *et al.,* 2006). Furthermore, stable flies have a labellum which is equipped with teeth and their proboscis is forward-facing, slender and sharp to assist in piercing skin (Todd, 1964; Patra *et al.,* 2018). These flies are also sexually dimorphic, and the compound eyes of male stable flies are closer together compared to females (Zeil, 1982). Collectively, these characteristics can assist in the identification of stable flies. However, as with all holometabolous insects, the conditions in which larvae develop can significantly affect their adult size and fitness (Baleba *et al.,* 2020).

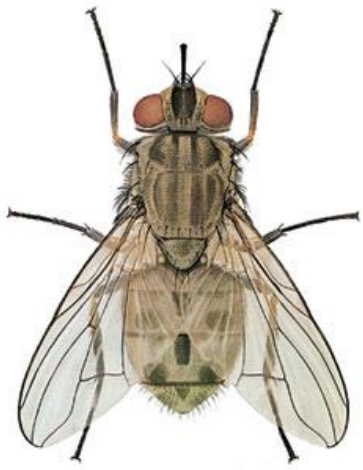

Figure 1. Female *Stomoxys calcitrans* L. (from Cumming, 1998).

There are four life-cycle stages: eggs, larvae, pupae and adults. Following their first blood meal, male stable flies can successfully inseminate females; this usually occurs around four days after

emergence and subsequently females start laying eggs at around eight days after emergence (Killough and McKinstry, 1965; Anderson, 1978; Morrison *et al.,* 1982). Gravid females lay clusters (~20-100) of white elliptical eggs within specific and individual oviposition substrates (Todd, 1964; Baleba *et al.,* 2020). These oviposition sites usually consist of putrefying organic materials, such as decaying grass, silage and hay, which are inherently found in close association with livestock (Meyer and Petersen, 1983). Once hatched, the translucent larvae bury into this medium and successively moult through three larval stages and subsequently pupariate into a reddish-brown pupation (Todd, 1964; Gilles *et al.*, 2005). Adults emerge 7-14 days after pupariation and can fly within one hour (Foil and Hogsette, 1994; Berry and Kunz, 1997). Notably, the duration of stable fly development is highly dependent on environmental conditions, such as temperature and availability of recourses (Florez-Cuadros *et al.,* 2019). For example, at 15°C the time required from the deposition of an egg to the emergence of an adult is 71 days, whereas it only takes 13 days at 30°C (Gilles *et al.*, 2005). Similarly, the average lifespan of stable flies varies, with wild flies living for approximately two weeks compared to over four weeks for those kept in a laboratory (Killough and McKinstry, 1965; Berry and Kunz, 1997).

Over the course of their life, stable flies usually take between one and three blood meals per day, each lasting around three minutes and imbibing 11-15 µL of the host's blood (Schowalter and Klowden, 1979; Harris *et al.,* 1974). These flies feed on a wide range of warm-blooded mammals, particularly bovids and equids (Patra *et al.,* 2018). As a synanthropic pest, common hosts also include domesticated animals, such as cats and dogs. They generally bite the thinner-skinned regions of their host due to easier penetration and higher density of capillaries near the surface. In bovids and equids, the front legs and underbelly are commonly bitten sites, whereas the tips of ears are more usually attacked in canines and felines (Yeruham and Braverman, 1995).

Due to their dependency on blood meals, stable flies usually aggregate in locations where hosts congregate, such as feed lots, water stations and outside shelters (Showler and Osbrink, 2015). As ectotherms, these flies are attracted to brightly illuminated surfaces, with high reflectance of ultraviolet light and iridescence, and rest on these surfaces during the morning to become active during the midday sun (Buschman and Patterson, 1981; Agee and Patterson, 1983). They usually employ a sit-and-wait predatory method, but they can make short distance (<1.6 km) flights in search of hosts and oviposition sites (Hogsette 1983; Showler and Osbrink, 2015). It has become evident that stable flies also perform long-distance dispersals; for example, one population of stable flies was found to have relocated 225km from inland Florida to the coast (Hogsette and Ruff, 1985). However,

such long-range migrations are usually considered to be passive movements, driven by weather. The weather can not only influence the distribution of these flies, but also their abundance (Lysk, 1993).

Stable fly abundance corresponds to several climatic factors and hence seasonal peak abundance often varies with location, depending on local conditions (Lysk, 1993; Machtinger *et al.*, 2016). In South-west England their numbers increase during summer months and peak in late August, early September (Parravani *et al.,* 2019). Despite Parravani *et al.,* (2019) finding no relationship between any climatic factors and their abundance, others have found that temperature and precipitation are strong predictors of stable fly prevalence (Lysk, 1993; Skovgård and Nachman, 2012). In a 13-year study in Nebraska, temperature and precipitation were found to be responsible for 72% of the variation in stable fly population, with populations peaking during the warmer season (Taylor *et al.,* 2017). In many agricultural environments increases in stable fly numbers during the summer months correspond to when livestock are more likely to be turned out and thus become easier targets for flies.

1.2 Veterinary Importance

1.2.1 Direct effects

The stable fly is one of the most problematic biting flies due to its irritability to hosts. By flying around and landing on their hosts, stable flies induce a range of defensive host behaviours, such as tail swishing, foot stamping, muscle twitching, head throwing, aggregating in groups and seeking protection by moving to other, less infested areas (Mullens *et al.,* 2017; El Ashmawy *et al.,* 2019; Kohari *et al.,* 2020). However, these behaviours come at a cost to hosts. For example, a host's energy expenditure is increased and their foraging ability and time, thus their food and energy intake are reduced (Dougherty *et al.,* 1993). It has been claimed that cattle increase their bite size and herbage intake in order to compensate for this reduction in foraging time, although there is little clear evidence to support this (Dougherty *et al.,* 1994). These defensive behaviours may also result in injury; the aggregation of cattle during 'bunching' behaviours, whereby individuals migrate centrally into a group to seek greater protection, can lead to increases in injury and heat stress (Wieman *et al.,* 1992). Avoidance behaviours may also inadvertently prolong the period of annoyance as feeding flies are interrupted before completing their blood meal and thus require multiple feeds.

The close proximity of stable flies can also increase the physiological stress experienced by their hosts (Colwell *et al.,* 1997). In dairy cattle, there is a linear relationship between the cortisol concentrations of a cow and the number of stable flies residing on the animal; cattle experiencing on

average 0 or 26 flies/day were shown to have cortisol concentrations of 2.5 ng/mL and 56 ng/mL, respectively (Vitela-Mendoza *et al.,* 2016). Furthermore, indicators of stress, including increases in heart and respiration rates as well as rectal temperatures, have been recorded in cattle exposed to 25 flies (Schwinghammer *et al.,* 1987). Incidentally, stable flies are attracted to volatile compounds found in breath, such as carbon dioxide and 1-octen-3-ol, thus increases in breathing rates could potentially increase attraction (Hieu *et al.,* 2014). Furthermore, the painful bite of a stable fly and associated loss of blood can further increase the physiological stress experienced by a host (Colwell *et al.,* 1997). The loss of blood is not limited to that imbibed by stable flies, there is also pooling of blood around the bite site due to stable fly probing. Together, elevated stress levels and amplified body movements, increase the energy expenditure of hosts and thus reduces their available reserves for growth, maintenance and reproduction.

During the physical act of feeding, stable flies excrete saliva which contains pharmacologically active molecules that inhibit blood clotting and increase vasodilation (Swist *et al.,* 2002). Components of this saliva may also initiate immunological responses, which can lead to immunosuppression and allergic reactions in susceptible animals. One study demonstrated that an intradermal injection of 2.4 mg of stable fly protein can cause immediate hypersensitivity in susceptible horses, and thus could play a role in the aetiology of sweet itch (Braverman *et al.,* 1983).

1.2.2 Indirect effects

Stable fly feeding may also have a number of indirect effects on their hosts. The wounds created by stable flies can become infected, as evidenced by the necrotising dermatitis on the tips of dog ears and exudative dermatitis on horses and cattle lower legs (Yeruham and Braverman, 1995; Urban and Broce, 1998). These lesions and cutaneous infections can secondarily aid the recruitment of other hematophagous parasites and increase the incidence of infections, such as myasis (Yeruham and Braverman, 1995).

Furthermore, stable flies may indirectly affect their host due to their role in the epidemiology of pathogens. The transmission of pathogens could be facilitated by the interrupted feeding habits of stable flies as they regurgitate the blood of their previous host at their new feeding site (Butler *et al.,* 1977). However, despite numerous studies, there is relatively little good data to support this vectoral role, despite many claims to the contrary. For example, Turell *et al.* (2010) concluded stable flies could act as mechanical vectors of Rift Valley fever virus as they are capable of transmitting the virus from highly infected to susceptible hamsters under laboratory conditions. However, these results have not

been confirmed in field populations. Similarly, the discovery of Trypanosoma DNA in stable flies in Nigeria is not definite evidence that they are mechanical vectors (Odeniran *et al.,* 2019) since the presence of pathogen DNA only in the blood meal shows only its presence not whether it can be transmitted. More convincingly, capripox viruses were shown to survive within stable flies for six days after ingestion and be transmitted to susceptible goats and sheep under experimental conditions (Mellor *et al.,* 1987). However, at present, there is no epidemiological evidence for the mechanical vectoral capabilities of stable flies in nature.

Female stable flies have also been reported as intermediate hosts of the nematode, *Habronema microstoma* (Traversa *et al.,* 2008). Despite including field experiments, this conclusion comes primarily from positive polymerase chain reaction samples, rather than definitive evidence of transmission of the nematode. To fully elucidate the potential veterinary importance of these flies, their role in the epidemiology of transmittable pathogens warrants further, more detailed investigation.

1.2.3 Economic impact

The effects of stable flies on their hosts have not only great veterinary importance but are also economically significant due to losses in yield. These economic consequences have been most extensively studied in cattle due to their commercial importance. Unfortunately, studies designed to quantify these effects are often inconclusive often because they use inappropriate controls (Shaw and Atkeson, 1943; Campbell *et al.,* 2001), unsuitable environmental chambers (Miller *et al.,* 1973) and include additional biting fly species in their analysis (Cutkomp and Harvey, 1958; Morgan and Bailie, 1980) and are thus not suitable for evaluation. Taylor and colleagues (2012) collated the results from reliable studies and estimated that as few as 10 flies/cow/day can cause significant economic losses. In US dairies with a high stable fly abundance, it was estimated that losses of 139kg milk/cow/year can be expected, which equates to $40 per animal (Taylor *et al.,* 2012). In the meat industry, individual cattle under stable fly attack can incur annual weight losses of 26kg, equalling $48 (Taylor *et al.,* 2012). In total, stable flies cost the United States of America cattle industry $2.2 billion annually (Taylor *et al.,* 2012). These estimates are based on US agricultural prices in 2009, and only relate to cattle and hence are difficult to extrapolate to other systems. Thus, more research on the economic effects of stable flies on a wider range of host animals would be of value.

1.3 Stable Fly Control

1.3.1 Chemical

Since their development and introduction, synthetic chemicals have become strongly integrated into nuisance fly control programmes for livestock. Feed-additive insecticides, such as the organophosphate tetrachlorvinphos, are widely used as they prevent larval development in manure. However, these treatments are often ineffective against stable flies because of the variety of oviposition sites that this species uses (Campbell, 1977). Alternatively, surfaces surrounding livestock can be treated directly with environmental insecticide preparations with the aim of discouraging oviposition and increasing mortality (Hogsette *et al.,* 1987). However, due to the transient nature of their oviposition media and tendency of larvae to bury, this method of application is often impractical, inefficient and environmentally damaging. At present, the most effective mechanisms for stable fly control are pour-on formulations, including organophosphates, permethrins and pyrethroids (Muraleedharan, 2005; Mottet *et al.,* 2018). However, due to short residual activities, most pour-on treatments require repeated application which is expensive and, therefore, is only justified during periods of high stable fly abundance (Foil and Hogsette, 1994).

The repeated application of synthetic chemicals can cause both environmental and health hazards, including non-target effects and the contamination of livestock products (milk and meat) (Gebremichael *et al.,* 2013; Pouokam *et al.,* 2017; Sands *et al.,* 2018). For example, synthetic pyrethroids can cause non-target effects on both terrestrial and aquatic organisms, including biologically important species, such as the dung beetle (Uddin *et al.,* 2016; Sands *et al.,* 2018). In addition to mortality, dung beetles exposed to these chemicals can show an array of sublethal effects, including reduced motility and impaired reproductive output (Sands *et al.,* 2018; Weaving *et al.,* 2020). The ecosystem services provided by dung beetles was estimated to save the cattle industry, in the United Kingdom, £367 million annually (Beynon *et al.,* 2015). As with the dung beetle, other important species are adversely affected by the routine application of insecticides and consequently there have been efforts to minimise the use of synthetic pesticides.

A range of alternative application methods have been developed which may have less environmental impact. For example, insecticide-impregnated ear tags are commonly used against flies on cattle (Hogsette and Ruff, 1986). However, as these devices rely on the self-grooming and movements of cattle for application, the neck and shoulders receive the greatest coverage and hence these devices are inadequate against leg biting stable flies (Beadleas *et al.,* 1977). The use of permethrin impregnated tail tags was investigated on dairy cattle and it was concluded that this mechanism was much more effective, eliminating stable flies within 24 hours (Hogsette *et al.,* 1987).

Despite this success, these tail tags have not been marketed due to their short residual activity and increasing incidence of insecticide resistance in flies.

The progressive development of pesticide resistance in stable flies means these routinely administered synthetic treatments are becoming ever more ineffectual in many parts of the world (Cilek and Greene, 1994; Pitzer *et al.,* 2010). Salem and colleagues (2012) investigated the level of resistance in stable flies collected from an organic and conventionally treated farm to six chemical treatments: cypermethrin, fenvalerate, permethrin, λ-cyhalothrin, deltamethrin and phoxim. Flies from the conventional farm were resistant to the five synthetic pyrethroids and the authors suggested using alternative organophosphate treatments (Salem *et al.,* 2012). However, the application of additional insecticides will increase resistance and enhance the potential for environmental damage caused by their application. Hence there is a need for new pest management approaches which are both effective and sustainable.

1.3.2 Mechanical

There has been considerable interest in the mechanical control of stable flies through pesticide-free traps (Taylor and Berkebile, 2006). The majority of these traps exploit the flies' optical attraction to polarised sunlight by coating reflective materials with an adhesive layer (Williams, 1973; Taylor and Berkebile, 2006; Turell *et al.,* 2010; Hogsette and Kline, 2017). Additional attractants, such as carbon dioxide, have been evaluated as complementary olfactory stimuli, but have been considered unnecessary as the equipment required for their production outweighs the additional gain in the number of flies caught (Cilek, 1999). The effectiveness of these traps has been demonstrated under a wide range of conditions. However, to manage a stable fly population effectively large numbers must be caught in close proximity to hosts and achieving this in large-scale agricultural settings is difficult (Ose and Hogsette, 2014; Hogsette and Kline, 2017; Hogsette and Ose, 2017). Hence, it is generally concluded that, while traps are effective for monitoring and supressing populations, they need to be used in conjunction with other techniques for population elimination (Hogsette and Kline, 2017).

1.3.3 Biological

Parasitoids, both naturally occurring and introduced, have been considered as alternative biological control strategies against stable fly infestations. However, their effectiveness varies greatly. On organic dairy farms in Denmark, a bi-weekly release of *Spalangia cameroni* distinctly reduced the number of stable flies per animal (Skovgård, 2004). However, when sentinel pupae of *Muscidifurax raptor* and *S. nigroaenea* were introduced into outdoor feedlots in Nebraska, even at fivefold the

recommended rate, there was no reduction in the stable fly population (Andress and Campbell, 1994). This variation can be explained by differences in climatic conditions and animal husbandry practices. However, a three-year study in Illinois showed between year variation in the effectiveness of *S. nigroaenea* and *M. raptor* at the same location, suggesting limited and inconsistent efficacy (Weinzierl and Jones, 1998). The level of parasitism provided by these parasitoids positively correlates with temperature, thus affecting their effectiveness across a season (Skovgård, 2004). Interestingly, individual parasitoid species are locally adapted to attacking stable fly larvae in different substrates and conditions, thus for optimum success, enhancing naturally occurring populations may be the most effective mechanism (Pitzer *et al.,* 2011). Therefore, for the appropriate use of parasitoids, there must be individual assessments and continued monitoring of effectiveness, which may be impractical and expensive.

1.3.4 Cultural

One of the most effective approaches to stable fly control involves adopting higher sanitation standards in agricultural areas (Hogsette *et al.,* 1987). Repelling and killing adult stable flies only causes periodic suppressions in the population, since developing eggs and larvae will subsequently emerge. Therefore, limiting the availability of oviposition media is an effective approach to a successful long-term pest management. This can be achieved by stacking the hay in dry places to ensure humidity is too low for stable fly eggs (Hogsette *et al.,* 1987). Furthermore, improving water and manure drainage systems can reduce the larval abundance in putrefying organic materials. Limiting the availability of oviposition media is one of the most important approaches to their control and should be used in conjunction with other mechanisms to prevent immigration from neighbouring sites.

1.4 Botanical Pesticides

Botanical-based pesticides have been utilised in agriculture for centuries (Isman, 2006). Before the advent and introduction of modern chemical pesticides, traditional methods of controlling and managing livestock ectoparasites were developed and many of these are still being used among indigenous communities around the world (Wanzala *et al.,* 2012). With the evident shortcomings associated with synthetic neurotoxic pesticides, the investigation of botanical alternatives warrants further investigation.

Considerable research interest has been focussed on the use of neem, rotenone, pyrethrum and essential oils (Isman, 2006; Isman and Grieneisen, 2014). Neem oil and seeds from Indian neem

tree, *Azadirachta indica*, are both of great interest due to their insecticidal activity. Neem oil has a physical mode of action which works synergistically with the oil's disulphides to achieve a lethal effect. However, neem seeds can function as an antifeedant as well as a moulting inhibitor (Isman, 2006). Rotenone is produced in the rhizomes and roots of tropical legumes and prevents energy production by acting as a mitochondrial poison (Hollingworth *et al.,* 1993). Pyrethrum is the insecticidal oleoresin extracted from the flowers of the Dalmatian chrysanthemum daisy, *Tanacetum cinerariaefolium*, which causes the rapid knockdown of insects due to its high pyrethrin concentrations (Corcos *et al.,* 2019). Finally, essential oils are volatile liquids which are a plant's natural defence mechanism against fungi, bacteria, insects and other herbivorous pests (Isman, 2006). The potential for essential oils to be used as a successful ectoparasite control agent will be discussed further.

1.4.1 Essential oils

Essential oils are volatile hydrophobic liquids made from a blend of 20-80 secondary metabolites of low molecular weight which are typically extracted from aromatic plants by steam distillation (Bakkali *et al.,* 2008). These oils are produced, stored and secreted by highly specialized tissues within vascular plants, such as glandular trichomes, which are specialised hair cells found on the leaves, stem and occasionally petals of aromatic plants (Markus and Turner, 2013). The secreted essential oils are usually characterised by high concentrations (20-70%) of two or three major terpenoid or terpene compounds as well as trace amounts of other aliphatic and aromatic components (Bakkali *et al.,* 2008). Gas chromatography–mass spectrometry (GC-MS) is frequently used to investigate the composition of these oils (Schmidt *et al.,* 2009; Najafian, 2016). This technique can be utilised as the gas chromatography separates molecules, which are then identified by mass spectrometry. Fortunately, the increasing cost effectiveness of this technique has allowed most studies investigating essential oils perform their own GC-MS prior to investigation (Isman, 2017). For example, Schmidt *et al.,* (2009), using GC-MS, established that the essential oil extracted from peppermint, *Mentha piperita*, contained over 40 compounds, with menthol and menthone comprising over 60% of the oil. Similarly, Nchu *et al.* (2012) established that Kenyan mint marigold, *Tagetes minuta,* contains high proportions of monoterpenes, with cis-ocimene and beta-ocimene being major components. Understanding the composition of essential oils is important as their pharmacological properties have often been attributed to the blend, particularly of their major components.

The biological activity of essential oils is broad ranging, including insecticidal (Kosgei *et al.,* 2014), growth inhibitory (Nchu *et al.,* 2012), antifeedant (Rajkumar *et al.,* 2019), repellent (Mkolo and Magano, 2007) and oviposition deterrent properties (Callander and James, 2012). The insecticidal

effects of essential oils have been most extensively studied on ectoparasites of medical and veterinary importance, including mosquitos, ticks, lice, mites and flies (Ellse and Wall, 2014; Benelli and Pavela, 2018a). Notably, these insecticidal effects have been documented across multiple life cycle stages. For example, exposure to 5% (v/v) lavender essential oil in N-lauroylsarcosine sodium salt, caused 100% mortality in adult and nymph chewing lice, *Bovicola ocellatus*, and inhibited all eggs from hatching (Sands *et al.,* 2016). The fact that essential oils have ovicidal, larvicidal and adulticidal effects may mean that fewer treatments could be required to eliminate target pest or parasite infestations.

Due to their volatility, essential oils can also act as deterrents or repellents (Ellse and Wall, 2014). For example, the essential oils from camomile, *Matricaria chamomilla*, camphor, *Cinnamomum camphora*, peppermint and onion, *Allium cepa,* were found to repel flies from water buffalo, *Bubalus bubalis*, for up to six days (Khater *et al.,* 2009). In addition, these oils can inhibit the natural behaviours of insects. Kenyan mint marigold essential oil (0.1 mg of neat oil) was shown to be able to deter 80.1% of brown ear ticks, *Rhipicephalus appendiculatus*, from their natural questing behaviour in *in vitro* tick climbing bioassays (Wanzala *et al.,* 2014). Similarly, gravid *Lucilia cuprina* delayed oviposition for 6 weeks when the only available media was wool treated with 5% (v/v) tea tree, *Melaleuca alternifolia,* oil (Callander and James, 2012). Lower concentrations of essential oils appear to be required to achieve a repellent effect compared to mortality (Wanzala *et al.,* 2012; Moyo and Masika, 2013). In combination, the insecticidal and repellent properties of essential oils make them comparable to several conventional control strategies.

As discussed, the efficacy of essential oils is often attributed to their major components; however, it has been suggested that minor constituents may also have important additive and synergistic effects (de Oliveira *et al.,* 2017). For example, after evaluating the repellent effectiveness of spindle pod, *Cleome monophyla*, oil against brown ear ticks, the authors concluded that all the components, including minor constituents, were required to achieve the greatest efficacy (Ndungu *et al.,* 1995). Trace elements in essential oils may also play an important role. For example, nerolidol (0.1%), had a repellent effect against brown ear ticks which was greater than all the major constituents of cat's whiskers, *Gynandropsis gynandra,* and the commercially available synthetic repellent, N,N-diethyl-toluamide (DEET) (Lwande *et al.,* 1999). Combinations of different essential oils and their compounds can also enhance the overall biological activity (Hieu *et al.,* 2010b). Thus, understanding the composition of an essential oil is fundamental in understanding its biological activity.

While most of the literature focuses on the composition and efficacy of essential oils, less is known about their mode of action. There is evidence that these oils can have both a contact and fumigant insecticidal effect. The hydrophobic nature of the oils means they are able to interfere with arthropod cuticular waxes and block spiracles which results in water stress and prevents gas exchange (Burgess, 2009; Ellse and Wall, 2014). This effect has also been seen in lice exposed to non-essential oils such as silicon (Talbert and Wall, 2012). However, unlike non-essential oils, exposure to essential oil vapour can also result in mortality, implying a simultaneous neurotoxic fumigant effect (Nchu *et al.,* 2012; Zhu *et al.,* 2012). There is evidence that essential oils can interfere with the central nervous system of insects, resulting in symptoms similar to those caused by synthetic insecticides, such as hyperextension of the appendages, paralysis and death (Table 1). These adverse outcomes can occur if essential oils are ingested or if they pass through the insect's spiracles or penetrate their cuticle (Zhu *et al.,* 2011; Callander and James, 2012).

These neurotoxic effects have mainly been attributed to three modes of action (Table 1) (Yeom *et al.,* 2015). The most extensively investigated method is the inhibition of acetylcholinesterase, the enzyme responsible for hydrolysing the neurotransmitter acetylcholine. Numerous essential oil components can competitively and non-competitively inhibit the acetylcholinesterase enzyme, in a dose-dependent manner, and subsequently cause the deregulation of nerve impulses (Table 1). However, it is unlikely that this is the primary route by which essential oils cause a neurotoxic effect because at low essential oil concentrations, where neurotoxic effects of essential oils have been recorded, there is often limited inhibition of acetylcholinesterase, and this inhibition is reversed quickly (López and Pascual-Villalobos, 2010; Anderson and Coats, 2012).

An alternative mode of action is the allosteric modulation of the gamma-amminobutyric acid (GABA)-gated chloride channels found in the post-synaptic neuron (Table 1) (Tong and Coats, 2010). The binding of particular essential oil components can initiate these chloride channels to open, thus facilitating unregulated neural impulses (Tong and Coats, 2010). However, the most convincing mode of action, due to its prolonged efficacy, comes from the ability of essential oil components to interfere with octopamine and tyramine (precursor to octopamine) receptors (Table 1) (Jankowska *et al.,* 2018). Octopamine is a multifunctional molecule in insects which has several biological roles, including a neurohormone, neurotransmitter and a neuromodulator (Orchard, 1982). Essential oil components are mainly agonists of octopamine receptors which initiate a cascade of effects, including increasing intracellular cAMP and calcium levels as well as protein phosphorylation and cause the dysregulation of the insect's nervous system (Enan, 2001).

Table 1. Three proposed neurological modes of action of essential oils and their components on insect nervous systems.

Mode of Action	Mechanism	Evidence of essential oils or components
Cholinergic system	Inhibition of acetylcholinesterase	1,8-cineole and terpinen-4-ol from tea tree (*Melaleuca alternifolia)* (Mills *et al.,* 2004). Camphor, E-anethole, fenchone, geraniol, (−)-linalool, S-carvone, γ-terpinene (López and Pascual-Villalobos, 2010). Carvacrol and nootkatone from Alaskan yellow cedar tree (*Cupressus nootkatensis*) (Anderson and Coats, 2012). Oriental sweetgum (*Liquidambar orientalis*) and valerian (*Valeriana wallichii*) (Park, 2014). Artemisia ketone, β- caryophyllene, β-phellandrene, camphene, camphor, cis-ocimene and estragole (Yeom *et al.,* 2015). Perilla aldehyde from peppermint (*Mentha piperita*) (Park *et al.,* 2016).
Gamma-amminobutyric acid system	Modulation of GABA receptors	Thymol from thyme (*Thymus vulgaris*) (Priestley *et al.,* 2003). Lemongrass (*Cymbopogon citratus*) (Costa *et al.,* 2011). Carvacrol, pulegone, and thymol (Tong and Coats, 2010).
Octopaminergic system	Agonists and antagonists of octopaminergic receptors	Eugenol and α-terpineol (Enan, 2001). Eugenol, cinnamic alcohol, and trans-anethole (Enan, 2005).

As different components in the essential oil can act simultaneously, the neurotoxic modes of action of these oils are not mutually exclusive. Encouragingly, the different compounds can work additively to increase the overall biological activity of an oil. Furthermore, the use of different cellular targets by different compounds could also slow down or even prevent the selection for resistance in pests. Firstly, individual compounds have different modes of action even when targeting the same

neurotoxic pathway (Jankowska *et al.,* 2018). For example, two monoterpenoid components found in tea tree, carvone and fenchone, target the acetylcholinesterase enzyme at different binding sites, thus even if an insect developed resistance against one compound, the other could still initiate a neurotoxic effect (López *et al.,* 2015). What is more, essential oil components target multiple neurotoxic pathways, with multiple modes of action within each pathway, and hence the likelihood of an insect developing resistance against all of these combinations is very unlikely. Due to their alternative modes of action, essential oils have been shown to be effective against organophosphate-resistant strains of tick (Costa-Júnior et al., 2016). These qualities make essential oils desirable alternatives to single compound synthetic pesticides.

In terms of the repellent effect, essential oil components may interact with the insect's olfactory system and either cause adverse reactions or disrupt normal function. Usually, a volatile odorant interacts with an olfactory receptor and co-receptor and consequently an action potential is initiated in the olfactory receptor neuron, and this relays the information to the antennal lobe (Andersson *et al.,* 2015). However, compounds found in essential oils may be allosteric agonists or antagonists of these receptors and hence could modulate the odorant receptor activity and disrupt the ability of the insect to detect scents. Bohbot and Dickens (2010) showed that the widely used insecticides, 2-undecanone, picaridin, DEET and ethyl butylacetylaminopropionate, inhibited specific olfactory receptors; DEET strongly inhibited *Aedes aegypti* AaOR8 receptor but caused no effect on AaOR2. The mechanism of repellence is a controversial topic as it is not fully understood, and thus future work should continue to elucidate the mechanisms involved. In the field, essential oils may also mask the hosts odour and hence disrupt the host-seeking behaviour of pests (Adenubi, *et al*., 2018).

Despite their advantages, there are limitations to the application of essential oils as botanical pesticides. Isman (1997) claimed that the sustainable cultivation of plant material for essential oils is a barrier to their commercialisation. One of the main issues is the requirement of large quantities of plant material for small oil yields (0.5-6.8%) and hence the cultivation of large monocultures (Zheljazkov *et al.,* 2013). However, over the last two decades, there has been pioneering work in metabolic engineering to improve essential oil yields and hence reduced the need for so much plant material (Mahmoud and Croteau 2001; Lange *et al.,* 2011; Wang *et al.,* 2016). Using monoterpene rich spike lavender, *Lavandula latifolia*, Muñoz-Bertomeu and colleagues (2006) overexpressed a gene encoding the 1-deoxy-D-xylulose-5-phosphate synthase (DXS) protein, which catalyses the first steps in the methylerythritol phosphate pathway which is the source of isopentenyl diphosphate, a terpene precursor. The upregulation of DXS resulted in increases in essential oil yield from leaves and flowers

by 359% and 74.1%, respectively, compared to wild type controls. Therefore, genetic engineering could assist in the biosynthesis of essential oils and increase yields which could in turn reduce the need for large monocultures. It should be considered however, that essential oils are often sold as natural alternatives, thus genetically modification may reduce their public perception and utilisation in organic farming practices.

Furthermore, the composition, quantity and quality of essential oils can vary considerably depending on the plant species, age, organ and vegetative cycle stage (Silvestre *et al.,* 1997; Perry *et al.,* 1999) as well as the climatic and soil conditions in which the plant has been grown and harvested (Holm *et al.,* 1997; Masotti *et al.,* 2003; Angioni *et al.,* 2006; Bakkali *et al.,* 2008). An assessment of 16 *Lippia kituiensis* samples, all from South Africa, revealed 5 different chemotypes: carvone, ipsenone, linalool, myrcenone, and piperitenone rich types (Viljoen *et al.,* 2005). Since efficacy is believed to be attributed to its composition, variation in chemotypes poses as an inherent problem in the ability to definitively attribute pesticidal or repellent properties to a particular plant species (Nchu *et al.,* 2012; Wanzala *et al.,* 2014). Hence, to ensure homogenous essential oil compositions, all variables must be controlled, and their composition must be assessed using gas chromatography and mass spectrometry (GC/MS). Characterisation of essential oils not only helps identify differences within species, but also, if an oil shows potential, other oils with analogous compositions can be investigated. At present, the International Organization for Standardization only standardises the essential of Australian tea tree, *Melaleuca alternifolia*, under the name *Melaleuca* terpinen-4-ol type (IOS, 2017). For the commercial production and utilisation of essential oils as botanical pesticides further standardisation regulations must be implemented.

The composition of an essential oil is also governed by the conditions in which it is extracted, stored and applied (Périno-Issartier *et al.,* 2013; Rowshan *et al.,* 2013). Due to their high proportions of terpenoids, essential oils are volatile substances which are highly susceptible to biodegradation (Turek and Stintzing, 2013). This degradation is associated with the interactions between compounds, primarily through autoxidation, which is enhanced by visible and ultraviolet light as well as temperatures which are too high or low (Misharina *et al.,* 2003; Misharina and Samusenko, 2008; Najafian, 2016). For example, lavender, *Lavandula officinalis*, oil kept at 25 °C for four months had significant changes to its chemical profile due to reductions in compounds of low molecular weight, including α-pinene, β-pinene, camphene and sabinene (Najafian, 2016). In comparison, lavender oil kept at 4 °C maintained its original chemical composition. In addition to environmental conditions, exposure to heavy metals can accelerate the rate of autooxidation and contribute to changes in oil

composition, thus these oils must be kept in inert plastic containers in at cold temperatures (Turek and Stintzing, 2013). Fortunately, unlike conventional treatments whereby sub-optimum levels can decrease the effectiveness of the solution and lead to the increased development of resistance, this is unlikely to occur for essential oils. Even if the composition of the essential oil has been altered, there are still numerous compounds, using several different modes of actions which could still be effective against the target species and the insect is still unlikely acquire resistance to these However, the resultant changes in an oil's composition can alter its efficacy and allergenic properties, which may be of particular significance if they are to be applied topically to animals as pesticides (Hagvall *et al.,* 2008; Pavela and Sedlák, 2018). Therefore, the lack of consistency and stability in essential oil composition is their principal limiting factor for commercial production.

The instability and volatility of essential oils also limits their environmental persistence and residual activity and hence repeated treatments may be required to deter persistent parasite challenges (Klauck *et al.,* 2014; Lachance and Grange, 2014). Attention has been focused on enhancing both the stability and residual activity of essential oils through the use of different excipients and mechanisms such as encapsulation (Maes *et al.,* 2019). This involves isolating biologically active molecules from external environmental conditions by coating them in a matrix wall (Zhu *et al.,* 2012). This matrix wall can be composed of natural, semi-synthetic or synthetic materials, but to align with the principles of botanical pesticides, natural coatings are preferred. Encouragingly, the encapsulation of peppermint oil in biodegradable chitosan nanoparticles increased its thermostability compared to pure forms by over two-fold (Shetta *et al.,* 2019). Furthermore, encapsulation of *Siparuna guianensis* with chitosan nanoparticles enhanced the duration of its larvicidal activity against yellow fever mosquitoes, *Aedes aegypti*, by slowing the release of biologically active compounds (Ferreira *et al.,* 2019). Unfortunately, encapsulated essential oils against livestock biting flies have only been assessed *in vitro* or in the field for short periods of time, thus it is unclear whether their longevity can be improved (Zhu *et al.,* 2010, 2014; Galli *et al.,* 2018).

Alternatively, the addition of specific excipients can increase the stability and residual activity of essential oils. Due to the hydrophobic nature of these oils, they are usually combined with water and an emulsifier to ensure a homogenised solution that can be easily applied to animals (Ellse and Wall, 2014). However, natural fixatives, such as liquid paraffin, salicylic acids and vanillin, have also been examined as potential excipients (Tawatsin *et al*., 2001; Oyedele *et al.,* 2002; Blackwell *et al.,* 2003). For example, when turmeric, *Curcuma longa*, essential oil was combined with 5% vanillin excipients the period of time in which it provided protection from *A. aegypti, Anopheles dirus* and

Culex quinquefasciatus significantly increased (Tawatsin *et al*., 2001). However, Kim *et al.* (2012) showed that the inclusion of vanillin with lemongrass essential oil caused notable decreases in the electroantennogram responses of *A. aegypti* and associated this with the fixative overly limiting volatilisation. Similarly, the efficacy of lavender and tea tree oils was reduced with coconut oil excipients (Sands *et al.,* 2016). Therefore, there is a balance between reducing the volatility and hence increasing the residual activity of the oil while maintaining its efficacy.

From an ecotoxicological perspective the volatility of essential oils may be beneficial as it could limit environmental contamination and bioaccumulation. The toxicity and persistence of these botanical pesticides are less than broad spectrum synthetic pesticides and hence could cause fewer residual effects (Muraleedharan, 2005; Khater *et al.,* 2009). Additionally, despite exploiting insect neurological pathways, there is interspecific variation in the effectiveness of essential oils which could lessen their non-target effects (Campbell, 1985; López and Pascual-Villalobos, 2010). For example, *S. guianensis* essential oil is an effective pesticide against green peach aphids, *Myzus persicae*, but has no adverse effect on the ladybirds, *Coleomegilla maculata* or *Eriopis connexa*, their natural enemies (Toledo *et al.,* 2019). Furthermore, essential oils are often considered safe to fish and mammals due to their insect specific pathways (Pavela, 2014; Pavela and Govindarajan, 2016). However, this is an area of contention, as the majority of studies document the efficacy of one essential oil against one insect pest and fail to incorporate other target and non-target species (Isman and Grieneisen, 2014). Therefore, future work should prioritise investigation of the environmental and non-target effects of these botanical pesticides.

In addition to environmental safety, understanding the vertebrate toxicity of essential oils is of importance if they are to be administered to livestock. The majority of essential oils are considered safe for use in humans, as shown by their widespread commercial use at low doses in food preparation, aromatherapy and cosmetics (Turek and Stintzing, 2013). However, specific components of oils can cause adverse reactions; for example, monoterpene ketones, such as cineole, camphor, pulegone and thujone are powerful convulsants (Burkhard *et al.,* 1999; Mossa *et al.,* 2018). As essential oil composition is so variable, even within chemotypes, all oils must be subject to individual characterisation and toxicological profiling. For instance, East Mediterranean sage, *Salvia libanotica*, harvested in the winter and spring contained different proportions of camphor and a,b-thujone, thus could exhibit different toxicities if topically applied to an animal (Farhat *et al.,* 2001). Furthermore, prolonged storage and the autooxidation of essential oils can increase their toxicity and skin-irritability, thus oils should be maintained in appropriate conditions and used before their use-by-date

(Hagvall *et al.,* 2008; Pavela and Sedlák, 2018). Therefore, extensive characterisation and a toxicological profiling of individual oils is essential prior to use on animals.

Even essential oils which have been regarded as safe have exhibited toxic effects when topically applied to animals, particularly at high concentration. A limitation to toxicological profiling is that the majority of studies are performed on cells *in vitro* or laboratory animals, such as mice and rabbits (Zhu *et al.,* 2009; Fouche *et al.,* 2017, 2019). These provide an insight into the toxicity of the oil, but in particular circumstances, these oils may react differently. For example, despite tea tree oil being advocated as safe for use in various cosmetic and medical treatments there have been adverse reactions to its administration, especially when at high concentrations (Yadav *et al.,* 2017). The application of 15 mL of 100% tea tree essential oil on the wing of a cockatiel, *Nymphicus hollandicus*, caused toxicosis, convulsions, vomiting, and resulted in a coma (Vetere *et al.,* 2020). Similar effects were documented in three Angora cats which had each been administered 60 mL of 100% tea tree (Bischoff and Guale, 1998). However, for uses as pesticides, essential oils are effective at low doses (<5%), thus it is unlikely adverse reactions would occur. Nonetheless, it is important to consider the safety of individual essential oils if they are to be applied to animals.

1.5. Insecticidal properties of essential oils against biting flies

Over the past three decades there has been extensive research into the insecticidal properties of essential oils. Within this literature, there has been a been a preponderance of studies of the vectors of human disease; hence mosquitoes have received a disproportionate amount of attention (Nerio *et al.,* 2010; Benelli and Pavela, 2018a). Within veterinary parasitology, ticks have been the most commonly studied ectoparasite, specifically species belonging to the genera *Rhipicephalus* and *Ixodes* (Benelli and Pavela, 2018a). Comparatively, biting flies have received limited attention (see Appendix I). In a literature search using Scopus, Benelli and Pavela (2018a) reported that 72% of research papers investigating the effectiveness of essential oils on biting arthropods were on mosquitos, 16% on ticks and only 2% on biting fly species from the families Ceratopogonidea, Simulidae, Tabanidae, Muscidae, Psychodidae and Glossinidea. Given the veterinary and economic importance of biting flies, more research should focus on understanding their susceptibility to essential oils.

1.5.1 Evaluating the efficacy of essential oils against biting flies

The methods used for assessing essential oil efficacy against flies are extremely variable. For the study of ticks, the Food and Agriculture Organisation (FAO) promotes the use of several well establish techniques, such as immersion and tick climbing bioassays (FAO, 2004). However, such

standardised techniques do not exist for flies and the World Association for the Advancement of Veterinary Parasitology (WAAVP) provides no specific guidelines for evaluating the efficiency of repellents against flies (Holdsworth *et al.,* 2006). As a consequence, comparing the efficacy of essential oil between studies is particularly challenging.

In immersion tests, insects are submerged in an essential oil treatment, usually for one to five min, and their consequent mortality is quantified, as well as the fecundity of treated females and egg hatchability (FAO, 2004; Callander and James, 2012). Similarly, aliquots of essential oils can be topically applied to flies (Zhu *et al.,* 2011). However, in the field it is unlikely a stable fly, at any lifecycle stage, would be directly treated in this way due to the mobility of adults and tendency for larvae bury into their developmental media. Therefore, assessing the toxicological consequences of contact with an essential oil treated surface is more appropriate.

The World Health Organisation (WHO) promotes the use of specific exposure kits to assess mosquito insecticide susceptibility, which can be adapted for flies (WHO, 2018). This is a standardised protocol which involves placing insects in tubes lined with impregnated filter papers and efficacy of the insecticide is taken as a measure of insect mortality at intermittent time intervals. However, there is a limited range of pre-impregnated filter papers available and the procurement of expensive specific equipment makes this technique inaccessible to many (Aïzoun *et al.,* 2013). There have however been several modifications to this experimental design, including placing flies in Petri dishes containing treated filter papers (Farnsworth *et al.*, 1997; Cossetin *et al.,* 2018). Alternatively, the Centre for Disease Control (CDC) endorses the use of bottle bioassays, in which the interior of a glass bottle is coated with a fine layer of the test compound and flies are subsequently introduced and their mortality recorded (CDC, 2011). Lastly, due to the volatility of essential oils, their toxicity can be assessed independently by exposing target species to the vapour of oils without contact. For example, flies can be held in a small cage within a container which contains a treated filter paper (Zhu *et al.,* 2011).

Due to the mobility of flies, many studies have focussed on assessing the repellent properties of essential oils. One method of assessment is through dual choice experiments which involve simultaneously presenting flies with treated or untreated options and recording their movement and behaviour; these options may include food sources or oviposition sites (Callander and James, 2012; Baldacchino *et al.,* 2013). However, due to the volatility of essential oils, their vapour is likely to

influence the behaviour flies in close proximity, and thus where the two options are presented simultaneously even the untreated option is may be affected by the presence of the essential oil.

A better approach for determining the ability of an essential oil to deter flies is to use no-choice experiments (Callander and James, 2012). These include skin bioassays, where test formulations are applied to the arms of volunteers and the time taken for starved flies to feed is recorded as the protection time (Hieu *et al.,* 2010b). This can also include treating a blood meal or oviposition site and recording the resultant behaviour of flies (Callander and James, 2012). However, there is considerable variation in the experimental design adopted in different studies, which can make comparison between experiments difficult. For example, variation in extraction techniques, excipients, assays, concentrations and time periods makes the replicability and evaluation of research in this area challenging (Ellse and Wall, 2014). The standardisation of methodologies and experimental design is imperative to allow comparison of essential oil efficacy between studies.

Furthermore, the results from *in vitro* studies cannot be extrapolated into the field as environmental factors can influence the biodegradation and volatility of essential oils and hence their efficacy (Turek and Stintzing, 2013). The majority of *in vivo* studies use a topical application of essential oil formulations to livestock as sprays or washes and then compare the number of flies found on the treated and control individuals (Khater *et al.,* 2009; Lachance and Grange, 2014). With all of these protocols, it is imperative that appropriate controls are used. In addition to a synthetic insecticide positive control, an excipient only and untreated negative control should be included. Furthermore, in topical application and immersion experiments, using a non-essential oil as an additional positive control is critical to allow the neurotoxic effect of an essential oil to be distinguished from the mechanical effect of oils *per se* (Ellse and Wall, 2014). Without the use of appropriate controls any effects observed cannot be attributed to the essential oil or compared to conventional treatments.

1.5.2 The use of essential oils against stable flies

Hieu and colleagues (2010a, 2010b, 2014) performed some of the first experiments investigating the use of essential oils as potential repellents against stable flies. Hieu *et al.* (2010b) assessed the repellent properties of 21 essential oils against stable flies using skin bioassays. Six human volunteers had 12.5 mg of pure essential oil, diluted in ethanol, applied to the back of their hands, at 0.5 mg/cm^2 and were subsequently exposed to 15 female stable flies. Essential oils from patchouli, *Pogostemon cablin*, clove bud, *Eugenia caryophyllata* and lovage root, *Levisticum officinale*, showed the greatest potential as repellents as they protected subjects from stable fly bites for 3.67,

3.50 and 3.36 h, respectively (Hieu et al., 2010b). Furthermore, mixtures of essential oils with tamanu, *Calophyllum inophyllum*, essential oil (0.25:2.0 mg/cm^2), provided elongated protection times (Hieu *et al.,* 2010b). Alone leverage root and tamanu oil provided protection for 1.13 and 0.56 h, respectively, whereas combined, this increased to 2.68 h, which exceeded the protection provided by DEET (2.20 h). Using the same methods, essential oils obtained from *Zanthoxylum piperitum* and *Zanthoxylum armatum* were assessed for their repellent properties (Hieu *et al.,* 2010a, 2014). At 0.4 mg/cm^2 *Z. piperitum* and *Z. armatum* treatments prevented 72% and 52% of stable flies from feeding for 90 min. However, this was significantly lower than the positive control DEET which maintained 100% repellency over this period (Hieu *et al.,* 2010b).

Citronella, *Cymbopogon citratus*, has also been assessed for its repellent properties against stable flies (Baldacchino *et al.,* 2013; Mottet *et al.*, 2018). In electroantennogram experiments, citronella initiated a strong response in stable flies, suggesting a behavioural response (Baldacchino *et al.,* 2013). In an experimental arena, the flight behaviour of stable flies, which were simultaneously exposed to one essential oil impregnated (0.1 mg/µL) blood-soaked feminine hygiene pad and one treated with hexane only (100 µL), were recorded over a 10 min observation period. Flies spent significantly more time around the untreated blood source, with nine individuals (37.5%) taking a blood meal. No flies fed on the citronella oil treated blood source (Baldacchino *et al.,* 2013). Furthermore, the authors recorded an overall decrease in the movement of flies over the observation time. However, no trial was performed with two untreated blood sources to determine the natural behaviour of these flies, therefore, the cause of reduced movement is uncertain.

Following this *in vitro* study, Mottet *et al.* (2018) investigated whether a citronella-based formula could reduce fly annoyance behaviours in horses *in vivo*. The formulation consisted of citronella oil (30 mL), distilled white vinegar (355 mL) and Avon Skin So-Soft® (118 mL), and when sprayed on the legs of horses significantly reduced the number of tail swishes and shoulder twitches performed per minute. Interestingly, pyrethrin spray (5%) did not significantly reduce these behaviours, suggesting the citronella formulation was more effective (Mottet *et al.,* 2018). However, the only control in the study was an untreated horse, thus the reduction in fly annoyance behaviours cannot be solely attributed to the presence of citronella due to the other components in the solution. Therefore, despite the commercial utilisation of citronella based essential oil products, there is limited evidence to support their use.

The most extensively studied essential oil against stable flies is catnip, *Nepeta cataria*, a herbaceous mint plant (Zhu *et al.,* 2009). The oil of catnip is rich in monoterpenoid nepetalactones, which have been documented for their bioactivity against numerous insects (Peterson *et al.,* 2002; Bernier *et al.,* 2005; Feaster *et al.,* 2009). Firstly, Zhu *et al.* (2009) concluded that the oil was safe as the results of broad-spectrum safety profiling were comparable to other Environmental Protection Agency approved repellents. However, when applied topically (0.5 mL of pure oil) to New Zealand white rabbits, all four subjects showed erythema within four days which persisted for the duration of the experiment. Therefore, despite being categorised as a safe oil, the skin irritant properties of catnip should not be overlooked. Subsequently, the insecticidal and repellent properties of catnip essential oil against stable flies were investigated. The topical application of catnip oil concentrations of 50 µg/µL achieved 100% mortality in adult stable flies, although concentrations below 12.5 µg/µL, caused negligible toxicity (Zhu *et al.,* 2011). This dose-dependent response was also evidenced in fumigant bioassays, as exposure to 100 µg/µL of catnip essential oil caused over 95% mortality whereas less than 20% mortality was observed in 10 µg/µL treatments (Zhu *et al.,* 2011). This study is valuable as the insecticidal effects of catnip against stable flies is investigated, whereas the majority of studies focus on their repellent properties alone.

In *in vitro* repellency bioassays, Zhu *et al.* (2009) showed that impregnating the membranes of citrated bovine blood-soaked feminine hygiene pads with 300 µL of 67 µg/µL of catnip oil prevented 96% of starved stable flies from feeding for 4 h. However, at lower concentrations of 6.7 µg/µL and 0.67 µg/µL, no significant repellent effect was recorded. Interestingly, subsequent experiments showed that 70% of flies engorged when presented with a food source treated with 0.67 µg/µL of catnip oil, a percentage comparable to the mineral oil control (Zhu *et al.,* 2012). Catnip oil has also been exemplified as a strong deterrence of oviposition as a catnip-treated barrier (0.1 g/mL) around oviposition media repelled 98% of gravid females for 6 h (Zhu *et al.,* 2012). Collectively, these experiments show the contact and spatial repellent properties of catnip oil when administered at higher concentrations.

The efficacy of catnip oil was examined *in vivo* in field trails where the application of 250 mL of 30% (v/v) water-based and 15% (v/v) oil-based catnip essential oil formulation onto the legs of cattle significantly lowered the number of residing flies for 5 and 6 h, respectively (Zhu *et al.,* 2012). However, it must be noted that the mineral oil control also had a significant repellent effect on stable flies, thus the efficacy of the oil-based formulation cannot be solely accredited to the essential oil component (Zhu *et al.,* 2012). Furthermore, in each trial, one front and one hind leg was treated with

the essential oil and the other was the control. However, due to the evident spatial repellency of catnip oil, the controls are not independent of the effects of the essential oil and thus are not an appropriate comparison. Despite these shortcomings in the experimental design of this study, the results suggested that even at high doses the *in vivo* repellent effect provided by catnip oil is short-lived. This is problematic as high doses are likely to be expensive and have associated safety concerns, especially considering the irritability of this oil to skin.

To try and increase longevity and efficacy, Zhu *et al.* (2014) encapsulated catnip essential oil. The capsules were composed from a pork skin gelatine wall matrix and a core which consisted of pure essential oil and mineral oil (1:1) (Zhu *et al.,* 2014). Oviposition media coated with 0.5 g of microencapsulated catnip contained 98% fewer eggs than the control. However, this effect disappeared within 48 h. In growth inhibition assays, where stable fly eggs were placed on a developmental media treated with 0.5 g of these gelatine microcapsules, only 0.6% of third-stage larvae matured and survived after 7 d (Zhu *et al.,* 2014). It was noted that the media treated with catnip oil contained significantly fewer microbial communities and it was hypothesised that the inhibition of larval growth may be a consequence of decreased food resources for larvae (Zhu *et al.,* 2014).

In conclusion, few essential oils have been examined extensively for their repellent efficacy against stable flies and even fewer for their insecticidal effects. Of those which have, there are often been limitations in the experimental design. Catnip essential oil has been the most extensively studied but its practical use in the field has not yet been demonstrated clearly.

1.6. Aim of this thesis

The overall aim of the work described here was to assess the insecticidal and repellent efficacy of essential oils against stable flies. The first aim was to select potentially valuable essential oils through a semi-quantitative literature search, based on studies which had examined efficacy against other biting flies. Then, *in vitro* bioassays were to be designed to investigate the toxicity of selected essential oils. Subsequently, the aim was to use behavioural bioassays to explore the repellent quality of the chosen essential oils.

Chapter 2

Insecticidal and repellent effects of lavender, *Lavandula angustifolia,* and tea tree, *Melaleuca alternifolia,* essential oils against stable flies

2.1 Introduction

Stable flies are important ectoparasites due to their ubiquitous distribution and close association with economically valuable livestock hosts (Foil and Hogsette, 1994). The most common approach to their control is through the use of synthetic pesticides such as organophosphates and pyrethroids (Muraleedharan, 2005; Mottet *et al.,* 2018). However, recently, the negative consequences and diminishing effectiveness of these conventional treatments has become evident and there has been growing interest in finding sustainable alternative control mechanisms, including plant-based repellents and pesticides. Repellents could be used as components of an integrated pest management approach in conjunction with improved sanitation and removal of oviposition site material (Hogsette *et al.,* 1987; Holdsworth *et al.,* 2006).

The first aim of this study, therefore, was to semi-quantitively assess the efficacy of essential oils previously tested on biting flies and determine which held the greatest potential. Based on this assessment, two essential oils would be chosen for further investigation to determine their insecticidal and repellent properties against stable flies. It was hoped that the results from this study would contribute towards our understanding of essential oils as stable fly control agents and assist in the development of formulations which could be used against a range of ectoparasites in the field.

2.2 Methods and materials

2.2.1 Stomoxys calcitrans

A stable fly colony was established at the University of Bristol using pupae obtained from a 30-year old laboratory colony maintained at MSD Animal Health Innovation (Schwabenheim, Germany). The flies were maintained in entomological cages (30 x 30 x 30cm) at 22 ±0.5°C with 40–45% relative humidity under a 18:6 light:dark photoscopic period. Adult flies were fed daily by placing 4 g cotton wool soaked in 5mL citrated bovine blood (100 mL of 4% sodium citrate/L) in their cage.

Blood was collected regularly from the School of Veterinary Science abattoir (Langford, Bristol). Bioassays were conducted under the same laboratory conditions as used to maintain the flies.

2.2.2. Essential Oils

A semi-quantitative analysis of primary literature evaluating the efficacy of essential oils against biting flies was conducted to determine which essential oils had the greatest potential. The relevant peer-reviewed literature was found by searching on Web of Science (v.5.31; 15.10.2019; https://clarivate.com/products/web-of-science/) using the key terms: “essential oil” or “extract” or “plant product” and “insecticidal” or “repellent” and “Ceratopogonidea” or “Simulidae” or “Tabanidae” or “Muscidae” or “Psychodidae” or “Glossinidea”. A database of 31 studies investigating 68 essential oils was compiled, and points were allocated to the oils based on the following criteria: repellent and/or insecticidal efficacy; concentration of essential oil; experimental design (controls, sample size and methods); and practicality (cost, availability, safety). Each criterion was scored out of five points. To determine the consistency of essential oils, the mean number of points allocated to each essential oil was calculated. Based on this analysis, lavender, *Lavandula angustifolia* and tea tree, *Melaleuca alternifolia*, essential oils were selected for further investigation.

Steam-distilled lavender and tea tree (100%) essential oils were obtained from a commercial source (Naissance Trading and Innovation, Neath, UK). To prevent thermo-degradation these essential oils were maintained at 5±1°C in complete darkness (Najafian, 2016). To achieve 5% (v/v) concentrations, the essential oils were diluted with absolute ethanol (≥ 99.8%; VWR international, France). Furthermore, absolute ethanol was used as negative control to distinguish effects caused by the excipient. DEET (20% v/v) (97%; Sigma-Aldrich, Gillingham, UK), diluted in absolute ethanol, was used as a positive control. For each experimental treatment and replication, fresh suspensions of essential oils were made to avoid concentration and composition differences caused by evaporation and biodegradation.

2.2.3 Insecticidal Bioassay

The insecticidal effect of each oil was examined using filter papers impregnated with the test formulations, in an adaptation of the WHO insecticide resistance protocol (Farnsworth *et al*., 1997; Cossetin *et al.,* 2018). First, filter papers (Whatman No. 1; 150mm diameter) were fully saturated with a 1mL aliquot of each treatment: 5% (v/v) lavender essential oil; 5% (v/v) tea tree essential oil; absolute ethanol (excipient only control). This produced a concentration of 0.283 μL/cm^2 of essential oil on the filter paper. Filter papers spent 5 min in a fume cupboard to allow the solvent to evaporate

before being placed into a 135mm diameter plastic Petri-dish. Simultaneously, one-week old stable flies, of mixed sex, were briefly chilled (-14°C) to inactivity, and ten randomly chosen flies were placed on each of the dry filter papers and the Petri-dish lid was secured in place. Live and dead flies were counted over a 2-min observation period at 15, 30, and 45 min and 1, 2, 4, 6 and 24 h post exposure. A fly was recorded as dead if no movement was detected during the 2-min observation time and no response was detected after agitation with a paintbrush. All tests were performed in triplicate, using 10 new flies and formulations for each replication.

2.2.4 Repellency Bioassay

A repellency bioassay was designed to determine whether essential oils could deter stable flies from feeding (Fig. 2.1). Pre-experimental observations indicated that one-week old stable flies generally alighted on the upper surface of their cage and hence the test apparatus was designed accordingly.

An olfactometer apparatus consisted of a 40 cm long vertical tube, constructed from plastic beakers and drinks bottle. A feeding attractant composed of 4 g of cotton wool soaked in 5 mL of citrated bovine blood, was placed on a mesh-ended plastic cup, which formed the feeding chamber at the top of the apparatus (Fig. 2.1). Immediately below the attractant was a funnel constructed using the neck from a 2 L plastic drinks bottle. The funnel was lined with filter paper (Whatman No. 1). The filter paper had a 2 cm diameter central circular hole to allow the movement of flies through the apparatus to the attractant. Immediately prior to a test, the filter papers were saturated with 1 mL of a test solution: 5% (v/v) lavender essential oil, 5% (v/v) tea tree essential oil, 20% (v/v) DEET (positive control), absolute ethanol (excipient only) or no treatment. Filter papers were then placed into a fume cupboard for 5 min to allow the ethanol solvent to evaporate and were then secured into the bottle neck. An airflow through the apparatus from top to bottom was created using an electric fan (5 V DC, 25x25x10mm, 5.95 m^3/h, 600 mW, Sunon LTD, Kaohsiung City, Taiwan) powered by a 6 V DC battery.

One-week-old stable flies, which had been starved for 24 h, were briefly chilled (-14°C) and randomly allocated into mixed-sex groups of 10 and assigned a treatment were then placed in the lower chamber of the apparatus. This was composed of a mesh-ended plastic cup (Fig. 2.1). To obtain a blood meal, flies would have to travel from the lower chamber, through a treated funnel, into the upper feeding chamber. Preliminary experiments, with untreated filter papers, had shown that usually all stable flies had reached the blood-soaked cotton wool and fed within 60 min; thus, this would be an appropriate length of time to determine if test formulations affected the feeding behaviour of

stable flies. Once the flies had been introduced into the apparatus, the number of flies which had passed through the treated tunnel into the upper feeding chamber were counted at 5, 15, 30, 45 and 60 min.

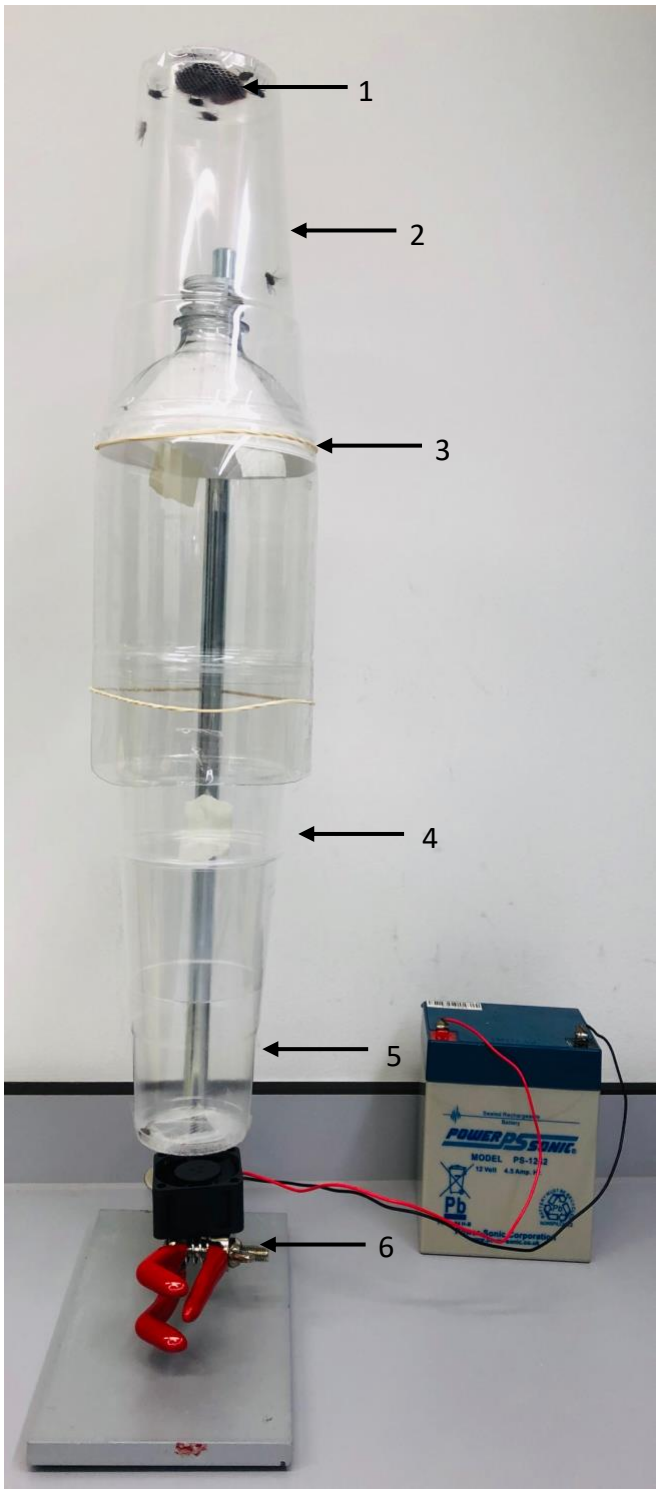

Figure 2.1. The experimental apparatus used to determine if essential oils were a feeding deterrent to *Stomoxys calcitrans*. (1) Blood soaked cotton wool placed on (2) a mesh-ended plastic pint cup which formed the upper feeding chamber. (3) The funnel was constructed from a 2L plastic bottle neck containing a treated filter paper. (4) A plastic pint cup connected to (5) a half-pint plastic cup with a mesh bottom which formed the entrance chamber. (6) Electric fan for airflow through the apparatus.

2.2.5 Statistical Analysis

All statistical tests were performed using RStudio (R Core Team, R Foundation for Statistical Computing, Vienna, Austria, Version 3.6.3, 2020), and a difference was considered statistically significant if $P<0.05$. Firstly, data was tested for homogeneity using Shapiro-Wilk test and normality of variance using a Levene's test. Both data sets were normally distributed and thus analysis of variances (ANOVA) were performed, followed by a Tukey post-hoc tests to determine differences between groups. For the insecticidal bioassay, the number of dead flies 15 min post-exposure was the response variable and treatment as the independent variable. This time frame was considered for analysis because if the essential oils were to be used in the field, they would need to be effective after a short period of exposure. In the repellency bioassays, the number of stable flies that reached the feeding chamber at 60 min was the response variable, with treatment as the independent variable. In each bioassay, recordings made at a single time only were used for ANOVA, to prevent the problems associated with non-independent observations. The time taken to achieve 50% mortality (LT_{50}) post exposure to treatment in insecticidal bioassays was determined for both lavender and tea tree essential oils using the dose.p function in RStudio.

2.3 Results

2.3.1 Essential Oils

From the literature search, 31 studies investigating the efficacy of 68 essential oils on over 15 species of biting flies were found (Appendix I). The plant family Lamiaceae had the highest representation (32.31%), followed by Asteraceae (13.85%), Myrtaceae (12.31%) and Rutaceae (7.69%). The essential oil most frequently tested was rosemary, *Rosmarinus officinalis* (5), followed by catnip (4), lavender (4) and tea tree (4).

Table 2. The number of points allocated to the top five performing essential oils.

Plant Species (Common name)	Average Number of Points Allocated to Each Category				Total Score
	Efficacy	Concentration	Experimental Design	Practicality	
Melaleuca alternifolia (Tea tree)	4	3.75	4.25	5	17
Lavandula angustifolia (Lavender)	3.75	3.75	3.5	5	16
Carapa guianensis (Andiroba)	3.5	3.5	5	3	15
Pelargonium graveolens (Pelargonium)	3.5	3.5	3.5	3	15
Nepeta cataria (Catnip)	3.5	3	3.75	4	14.25

2.3.2 Insecticidal Bioassay

In the analysis of the insecticidal efficacy of essential oils, even after only 15 min, stable fly mortality significantly varied between treatments (ANOVA, F4=19, P<0.001); significantly more stable flies died after exposure to filter papers impregnated with 5% (v/v) lavender essential oil (Tukey HSD, p<0.05) and 5% (v/v) tea tree essential oil (Tukey HSD, P<0.001), compared to the excipient-only ethanol controls (Fig 2.2). However, the mortality caused by exposure to lavender or tea tree essential oil was not significantly different (Tukey HSD, P=0.64). On average 3 ±0.58 and 3.67 ±0.67 flies died when exposed to lavender and tea tree oils, respectively, whereas no flies died when exposed to the ethanol control for 15 min (Fig. 2.3). The LT_{50} for lavender and tea tree essential oils were 54 and 51 min, respectively. Lavender essential oil caused 100% stable fly mortality within 4 h and tea tree within 6 h.

2.3.4 Repellency Bioassay

After 60 min in the apparatus the number of stable flies that reached the end chamber containing the blood varied significantly (ANOVA, F_4=19, P<0.001). Subsequent multiple comparison tests showed that the number of flies that passed the filter papers impregnated with lavender and tea tree essential oils were significantly less than untreated controls (Tukey HSD, P<0.001) and excipient-only ethanol controls (Tukey HSD, P<0.01). After 60 min, when exposed to untreated or excipient-treated filter paper funnels, a mean ± standard error of 9.67 ±0.33 and 7.67 ±0.33 flies passed into the feeding chamber, respectively, whereas only 1.67 ±1.67 and 1 ±0.58 flies had done so when the filter papers were impregnated with 5% lavender or tea tree essential oil, respectively. Only 3.67 ±0.67 flies

passed the filter paper when impregnated with DEET. The number of flies that passed the filter papers impregnated with the lavender (Tukey HSD, P>0.05) or tea tree (Tukey HSD, P>0.05) oils compared to the positive control DEET was not significantly different. However, the number of flies that passed the DEET impregnated filter papers was not significantly different from that observed with the ethanol negative controls (Tukey HSD, P>0.05).

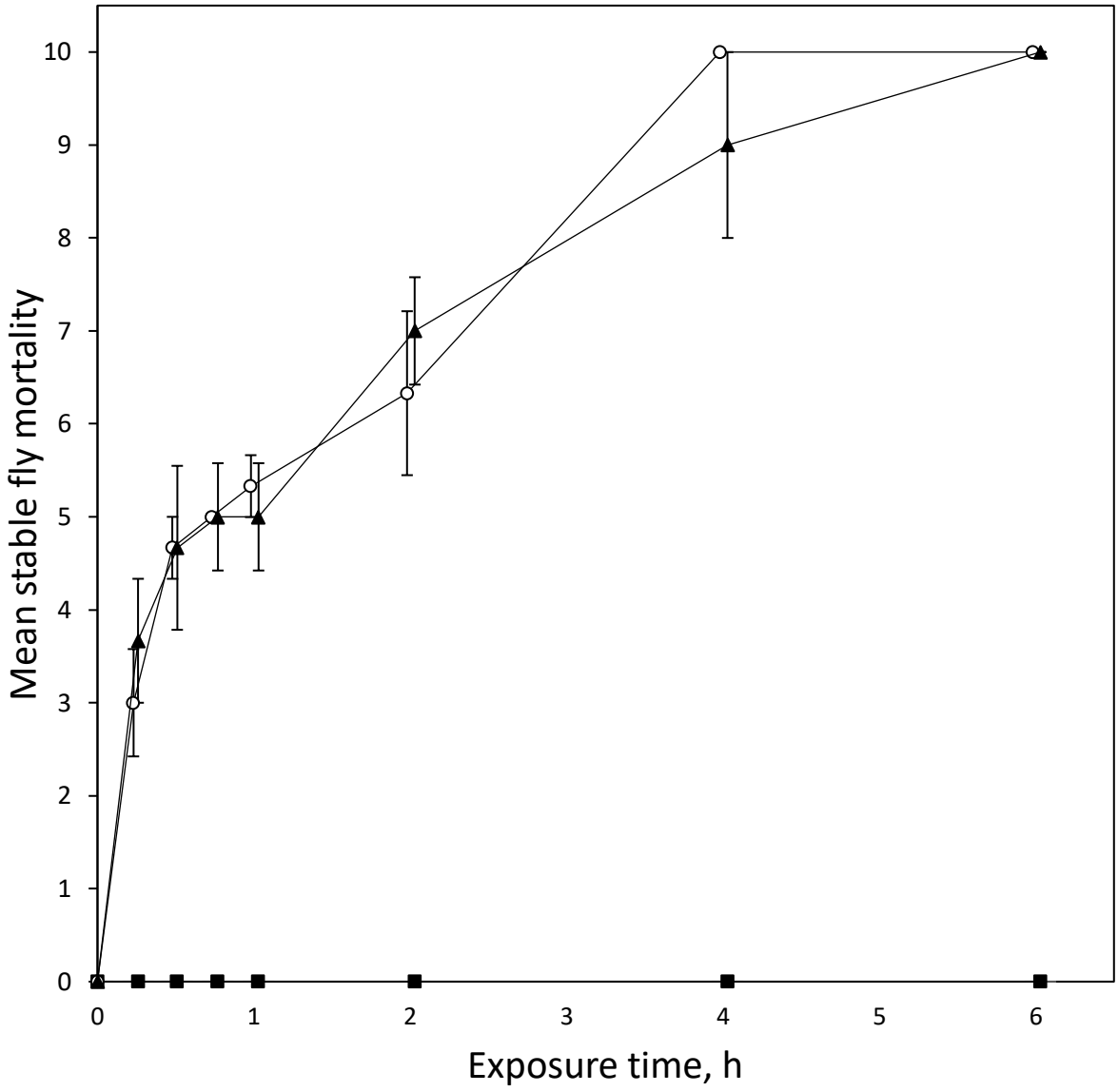

Figure 2.2 Mortality (mean ±SE) of *Stomoxys calcitran* at 15, 30 and 45 min and 1, 2, 4, and 6 h post-exposure to filter papers impregnated with 5% (v/v) lavender essential oil (○), 5% (v/v) tea tree essential oil (▲) and absolute ethanol excipient-only negative control (■). Points have been offset and joined for clarity.

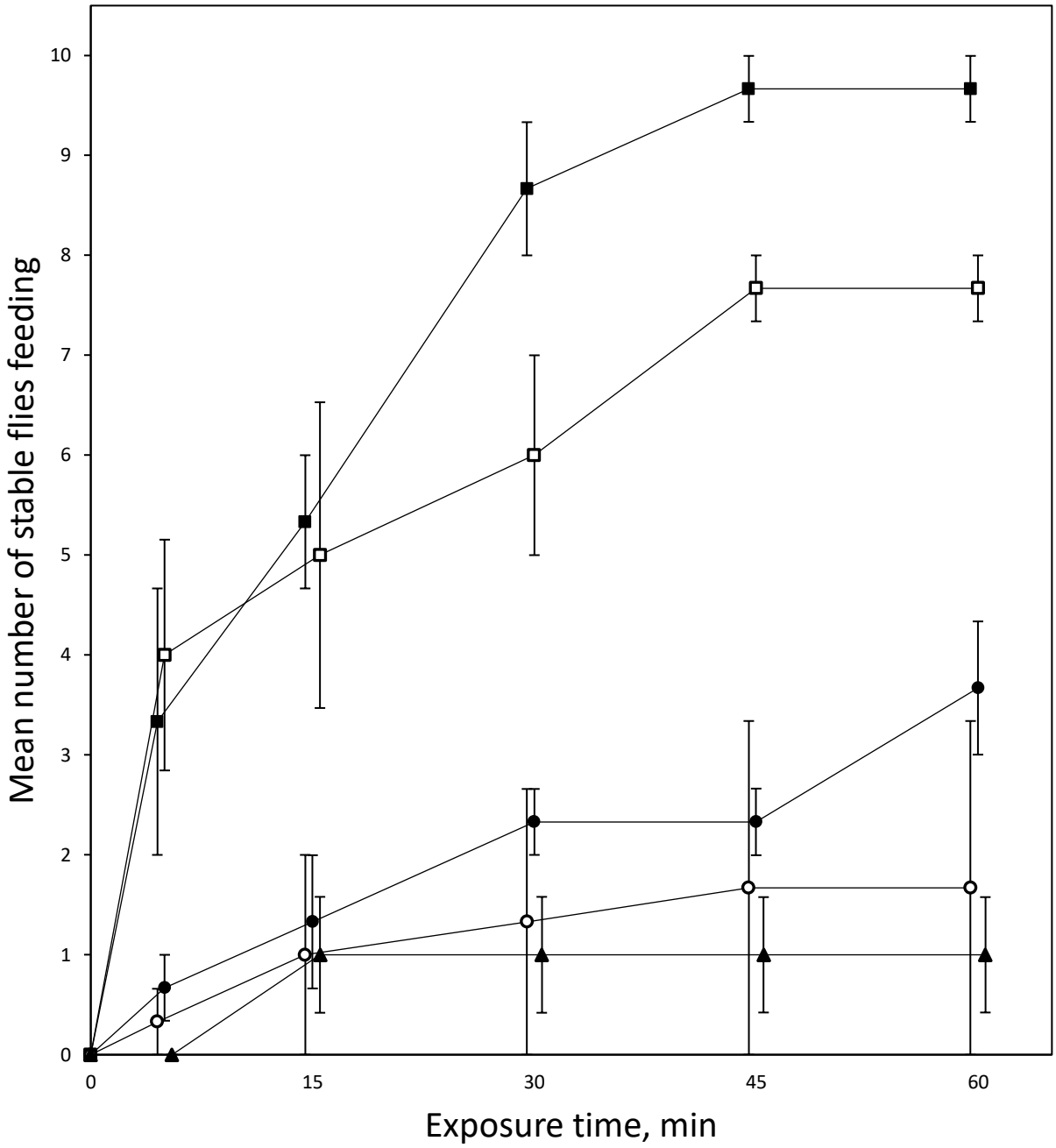

Figure 2.3 The number of *Stomoxys calcitrans* (mean ±SE) that reached the end chamber of an olfactometer containing blood-soaked cotton wool after passing a filter paper funnel impregnated with 5% (v/v) lavender essential oil (○), 5% (v/v) tea tree essential oil (▲), DEET (20% v/v) positive control (●), absolute ethanol excipient-only negative control (□) and untreated negative control (■) at baseline, 5, 15, 30, 45 and 60 min. Points have been offset and joined for clarity.

2.4 Discussion

Exposure to filter papers impregnated with either 5% lavender or tea tree essential oil caused significantly greater stable fly mortality than the control, even within 15 min, and achieved 100% stable fly mortality within 4 and 6 h, respectively (Fig. 2.2). The very low mortality in the excipient only controls, even after 24 h, provides a high degree of confidence that this mortality was a consequence of the essential oils (Fig. 2.2). The toxicity observed here appears to be greater than that recorded for catnip; when exposed to catnip oil, even at higher doses (0.2 mg/μL), 100% stable fly mortality was not achieved and 20% of flies originally recorded as dead recovered (Zhu *et al.,* 2011). Here, doses equivalent to ~0.044 mg/μL were sufficient to give 100% mortality and recovery was not observed (Naissance, 2020). The rapid knockdown and mortality achieved by lavender and tea tree oils shows their insecticidal potential against stable flies.

The results of this study are in accordance with previous biting fly experiments. Using a similar bioassay, Cossetin *et al*. (2018) showed the efficacy of lavender oil on the calliphorid *Chrysomya albiceps,* as concentrations of 0.1 mg/cm^2 caused 100% mortality within 2 h. Similarly, in adaptations of the CDC bottle bioassay, 0.2% (v/v) of lavender oil was shown to cause 100% mortality of *Lucilia sericata* within 5 min (Khater and Geden, 2018). Tea tree has also been shown to be toxic to biting flies; the topical application of 1% (v/v) tea tree oil killed 100% of horn flies, *Haematobia irritans*, within 3 h and, in feeding assays, 2.5% (v/v) concentrations caused 100% mortality of *Lucilia cuprina* second stage larvae (Callander and James, 2012; Klauck *et al.,* 2014). The variation in concentrations required to achieve mortality in different species may be a consequence of interspecific variation in fly size, pilosity, and physiological susceptibility and differences in experimental design.

The insecticidal efficacy and mode of action of essentials oils can be influenced by the experimental design of the study. For example, the fumigant toxicity of essential oils is associated with their vapour pressure and this can be influenced by the excipient and experimental arena used (Ajayi *et al.,* 2014). Here, essential oils were mixed with very volatile ethanol and the Petri-dishes formed small closed chambers, thus flies would have been exposed to high concentrations of oils in their vapour phase (Sfara *et al.,* 2009; Koutsaviti *et al.,* 2018). Previous studies have shown that the effectiveness and residual activity of oils is greater in closed chambers compared to open ones (George *et al.,* 2008; Sands *et al.,* 2016). Therefore, the mortality observed here can be mainly attributed to the absorption and inhalation of essential oils in their vapour phase (Cossetin *et al.,* 2018). Analysis in an open environment would be an appropriate next step to evaluate their effect in the field.

The equally rapid adulticidal effect caused by exposure to lavender and tea tree essential oils here is likely to have been due to their high concentrations of oxygenated compounds. Lavender contains a high proportion of linalyl acetate (43-48%), linalool (28%-34%) and 1,8-cineole (18%-24%) and tea tree oil consists of high proportions of terpinen-4-ol (35-48%) and 1,8-cineole (10%) (Najafian, 2016; ISO, 2017). Papachristos and colleagues (2004) found that essential oils containing higher proportions of oxygenated monoterpenoids exhibited increased insecticidal activity against the bean weevil, *Acanthoscelides obtectus*, thus this shared characteristic can help explain the effectiveness of lavender and tea tree oil. More specifically, these monoterpenoid compounds have been found to interfere with insect acetylcholinesterase and GABA receptors and result in the deregulation of the neuromuscular system, ataxia and insect death (Table 1). One of the most powerful inhibitors, 1,8-cineole, can cause 50% inhibition of acetylcholinesterase at doses as low as 0.015 mg/mL (Dohi *et al.,* 2009). Furthermore, minor components of these oils act synergistically to improve efficacy. For example, *α*-Pinene, a minor component of lavender (2.3%), is thought to work synergistically with 1,8-cineole to increase inhibition of acetylcholinesterase (Savelev *et al.,* 2003; Najafian, 2016). Collectively, the major and minor components of these oils are responsible for their efficacy.

Both lavender and tea tree essential oils prevented flies from passing impregnated filter papers within the olfactometer, and this suggests that both show promise as botanical stable fly repellents. Both oils were able to deter more flies from the food source than 20% DEET, a commercially available repellent recommended by the WHO to be used as a positive control when assessing new repellents (WHO, 2009). Furthermore, tea tree consistently repelled more flies from the feeding chamber than lavender oil, and with lower variation. Both of these oils showed great potential as botanical repellents. By definition, botanical repellents are natural substances which stimulate an avoidance response from their target species (Zhu *et al.,* 2015). This can be further categorised as either contact or spatial repellents, whereby contact repellents cause adverse reactions in target species post contact, and spatial repellents work in their vapour phase as volatile components are detected by the insect's olfactory sensilla and initiate an avoidance behaviour before contact (Achee *et al.,* 2009). Here, stable flies were observed flying away from the treated filter papers, before contact was made, implying a spatial repellent effect. Furthermore, the design of the bioassay used here suggests that the repellent behaviour was not likely to have been associated with an adverse reaction to the ingestion of the essential oil (Zhu *et al.,* 2012). Spatial repellence is a particularly useful quality in the field as a treatment could prevent stable flies coming in close proximity to hosts and hence avoid defensive host behaviours and the consequences associated with them (Section 1.2). Further

investigation of the spatial repellent properties of these oils, using electroantennogram analysis, may be of value (Hieu *et al.,* 2014).

In comparison to other essential oils used against stable flies, lavender and tea tree show greater promise as practical botanical pesticides, not only because of their great efficacy but also because these oils are considerably less expensive (Hieu *et al.,* 2010; Zhu *et al.,* 2011; Naissance, 2020). What is more, not only are these oils effect against stable flies, their repellent qualities have been evidenced against multiple biting fly species. For example, 1 µg/µL of lavender oil in hexane repelled 93.7% of flies for 4 min (González *et al.,* 2014). Furthermore, through a series of *in vitro* and *in vivo* experiments, formulations of tea tree oil (5% (v/v)) have successfully repelled horn flies from feeding for up to 24 h (Klauck *et al.,* 2014, 2015). Tea tree oil (3% (v/v)) also prevented oviposition in *L. cuprina* for 6 weeks (Callander and James, 2012). Encouragingly, all of the doses are low and thus a treatment of 5% lavender or tea tree appears likely to be an appropriate field dose to facilitate insecticidal and repellent effects against a variety of biting flies.

The final aim of the work to be undertaken as part of this research project was to conduct an investigation of the efficacy of these oils when applied to donkeys in the field at the Donkey Sanctuary farm in Devon. However, due to the COVID-19 outbreak this work was not possible. In the field, variable temperatures, ultraviolet light, wind and rain, may all increase the biodegradation of essential oils and reduce their efficacy and residual activity (Turek and Stintzing, 2012). Nevertheless, the repellent qualities of these oils have been examined in the field against other fly species. For example, the application of 5% (v/v) concentrations of lavender and tea tree significantly reduced the number of flies alighting on pastured cows for up to 5 and 24 h, respectively (Klauck *et al.,* 2014; Lanchance and Grange, 2014). Consequently, it can be concluded that both lavender and tea tree show promise as effective and sustainable control strategies against stable flies, and therefore they warrant further *in vivo* investigation to fully elucidate their potential in the field.

Chapter 3

General Discussion

3.1. General Discussion

Lavender and tea tree belong to the Lamiaceae and Myrtaceae family, respectively, which are among the most widely studied families for their pharmacological properties (Benelli and Pavela, 2018a). Lavender is an aromatic shrub native to the Mediterranean and the flowers produce a colourless oil with a strong floral fragrance (Cavanagh and Wilkinson, 2002). Comparatively, tea tree oil is usually pale yellow in colour with a distinct camphoraceous odour and is extracted from the tea tree plant which is native to East Australia (IOS, 2017). Both of these oils have been used as ethnobotanical therapeutic agents for centuries and with the increasing popularity in botanical alternatives, they have become of great interest over the last few decades (Cavanagh and Wilkinson, 2002; Yadav *et al.,* 2017). In Chapter 2, the insecticidal and repellent properties of lavender and tea tree essential oils were demonstrated against stable flies and both showed promise as natural alternatives to conventional synthetic insecticides.

For the use of these oil-based treatments in animal husbandry, they must be effective, safe, easily applied and economically viable. Synthetic neurotoxins are usually effective against a broad range of ectoparasites and hence, if essential oils are going to be considered as a viable alternative to synthetic pesticides, they must provide protection against a similar range of target species (Campbell, 1985). Encouragingly, the results from numerous field and laboratory experiments have shown efficacy of both lavender and tea tree against an array of veterinary important ectoparasites, including flies (see Appendix I) lice (James and Callander, 2012; Ellse *et al.,* 2015), mites (Mägi *et al.,* 2006) and ticks (Périno-Issartier *et al.,* 2010; Pazinato *et al.,* 2014). For example, Ellse and colleagues (2016) showed that two weeks after hand spraying donkeys with a 5% (v/v) lavender and tea tree formulation, the number of *Bovicola ocellatus* found on treated individuals decreased by 78%. Similarly, Mägi and colleagues (2006) showed that four weeks after treating pigs with 1% (v/v) tea tree emulsions, their sarcoptic mange mite, *Sarcoptes scabiei*, intensity of infection decreased by over 98%. The broad-spectrum efficacy of lavender and tea tree means they are likely to be viable alternatives to conventional neurotoxic treatments. Additionally, it is unlikely that an insect will acquire resistance to essential oil treatments due to their complex modes of action (see section 1.4). This is not only beneficial for the long-term efficacy of the treatment, but it also means that unlike with conventional

treatments, where targeted application is required to reduce the risk of resistance, essential oils could provide a year-round, long-term solution to prevent infestations from several parasites.

Lavender and tea tree essential oil also have antibacterial and fungicidal properties which could make them advantageous over conventional neurotoxins. Previous work has shown that concentrations of 0.013% and 0.5% (v/v), of lavender and tea tree oil, can initiate bacterial cell death (Cox *et al.,* 2000, 2001; Sienkiewicz *et al.,* 2014). More specifically, the lipophilic monoterpenoid components of these oils can affect the structural and functional properties of a bacterial membrane and consequently cause the dysregulation of intercellular homeostasis and inhibit cell respiration (Sikkema *et al.,* 1995; Cox *et al.,* 2000, 2001). Furthermore, terpinen-4-ol, linalool and 1,8-cineole have been shown to be effective fungicides at concentrations below 0.25% (Hammer *et al.,* 2003). As these compounds are highly represented in lavender and tea tree oils, they too could have useful fungicidal effects. Therefore, the topical application of these oil formulations may not only reduce ectoparasite numbers but could also improve the dermal health of the treated animal. What is more, these pharmacological properties have been demonstrated at low doses, thus 5% concentrations would be likely to provide adequate control against a wide range of ectoparasites, bacteria and fungi.

Lower concentrations of essential oils are beneficial as they are associated with minimised safety concerns. This is of particular interest if formulations are to be topically applied to animals which partake in self-grooming activities. Previous work has shown no skin irritability when 5% concentrations of lavender and tea tree essential oils have been topically applied to livestock and companion animals (Lachance and Grange 2014; Klauck *et al.,* 2014; Ellse *et al.,* 2016). Furthermore, the oral toxicities of lavender (LD_{50}: >2 g/kg) and tea tree oils (LD_{50}: 1.9–2.6 ml/kg) are below that of conventional insecticides (Russell, 1999; Mekonnen *et al.,* 2019; Cantalamessa, 1993). However, due to the lipophilic nature of essential oils, transdermal absorption can occur and thus residues of oils may accumulate in the muscles of treated animals (Herman and Herman, 2014). At present, no work has been conducted to elucidate the potential tainting of animal products, such as milk and meat, when oils are topically administered. However, it is unlikely to be a significant issue as Rivaroli *et al.* (2016) showed that the inclusion 3 g/animals/day of essential oil blends into the feeding regime of crossbred bulls had no effect on the chemical composition of their meat, thus tainting is improbable but specific analysis into the effect of topical application is required. Consequently, at present, these oil treatments can only be safely recommended for use on companion animals. Future work can focus on the commercialisation of impregnated tail tags for cattle, although it is likely these will only be

effective against flies and not provide simultaneous protection against permanent parasites (Hogsette *et al.,* 1987; Juan *et al.,* 2011).

Within companion animals, equids are readily attacked by stable flies and are hosts to numerous other ectoparasites, thus are prime candidates for these botanical treatments (Patra *et al.,* 2018; Karasek *et al.,* 2020). Several botanical treatments have already been commercialised for this market and hence there is an acceptance for these products within this sector which could be utilised. The essential oil formulations could be applied using the hand spray method employed by Ellse *et al.* (2013, 2016) for the treatment of donkeys. In the latter study, 2 mL of essential oil formulation was to be applied per kg body weight (to the nearest 50 kg) of the animal. Therefore, for an average size donkey, 400 mL of the solution was to be sprayed onto the individual during routine grooming practices. This simple and convenient spray technique is analogous to current treatments and hence could easily be introduced as an alternative. Furthermore, similar application methods have been shown to be operational on other animals which are targeted by stable flies, such as dogs (Goode *et al.,* 2018).

In terms of costs, the extraction of essential oils from aromatic plants is an expensive process due to the specific equipment required for distillation and the low oil yields (0.5-6.8%) obtained from plant material (Zheljazkov *et al.,* 2013). Fortunately, due to the popularity of lavender and tea tree essential oil in the food, cosmetic and natural health industries, they are commercially produced and hence are among the most affordable oils (Naissance, 2020). If the same application methods as Ellse *et al.* (2016) were used, based on current trading prices and already commercialised products, it would cost between £6.40 and £20 per treatment per animal, depending on excipients used (Agrient Limited, 2020; NAF UK, 2020). For high value animals such as equids, this is comparable to many of the conventional synthetic treatments used (e.g. Tri-Tec 14™, Farnam and NAF-Off DEET Power Performance, NAF). However, these costs would be inhibitory for use on livestock due to the increase in scale of use. The principle of using endemic botanical-based pesticides may be particularly attractive in less economically lucrative countries, as endemic plant species can provide a sustainable alternative to high cost synthetics, but at present this is not possible. Therefore, to allow the use of essential oil-based products on a greater range of animals and globally, there should be a continued focus on improving the oil yield through biotechnology and reducing the cost of the extraction process.

Isman (2006) has argued that the limited residual activities of essential oil-based formulations could inhibit their commercialisation. Their short period of effectiveness is less problematic in the

control of permanent ectoparasites, such as *B. ocellatus,* as one treatment could eliminate an entire parasite population if hosts are treated simultaneously and the risk of immigration was minimal (Ellse *et al.,* 2015; Sands *et al.,* 2016). However, to afford continuous protection against parasites with free-living stages, such as stable flies, a prolonged efficacy is fundamental. Therefore, if only effective over a short period, higher application frequencies may be required, and this may result in annual costs exceeding that of conventional treatments. However, in equid husbandry, numerous synthetic treatments require daily application including DEET, and hence the short residual activities of essential oils may be less problematic (Herholz *et al.,* 2016). In the present study, both tea tree and lavender significantly deterred starved stable flies from a blood source for one hour. However, due to the laboratory conditions and short time frame this data cannot be used to estimate their residual activity in the field. To more effectively quantify the residual activity of these essential oils in the field, *in vivo* studies, whereby the oils are applied to the animal hide, must be performed.

The excipient used for essential oil application can have a profound effect on the efficacy and residual activity of the treatment. Firstly, the design of the formulation can alter the hydrophobicity and improve its penetration into the coat of the animal and hence its residual activity. For example, James and Callander (2012) assessed the efficacy of tea tree oil against *B. ovis* on sheep and showed that the excipient used, which consisted of water, oleic acid and ethoxylated castor oil, assisted in the penetration of the essential oil into the wool. The authors also claimed that even after several weeks, the tea tree odour could be detected; this prolonged period of activity would be beneficial when an animal is under repeated challenge from stable flies. Furthermore, previous work has shown that different excipients can have a significant effect on the transdermal penetration of drugs and their distribution throughout the skin (Mills *et al.,* 2005; Mills, 2007). Therefore, future work should continue to investigate the effectiveness of essential oils in combination with different excipients *in vivo* as this may help improve their residual activities and prevent transdermal absorption and hence assist in their commercialisation in different sectors.

Despite the research into essential oils and their pesticidal properties, commercialisation of such formulations is limited. Before their use as medicines, these bioinsecticides require regulatory approval. In several countries, botanical pesticides are not distinguishable from conventional treatments and hence have to go through the same expensive regulatory processes (Isman, 2006). Due to the smaller market and profit margins for botanical pesticides, the cost of this process of registration could be inhibitory. However, the United States of America have exempted several essential oils from registration due to their popularity in the cosmetic and food industry and hence oil-based pesticides have been commercialised for over a decade. Similarly, the European Union has

excused essential oil-based formulation from registration if they are not for human use and consequently, in the past six years there has been an increase in the availability of botanical pesticides for companion animals and livestock (Isman, 2019). Therefore, due to their current acceptance in the European Union, it is probable that a new essential oil product would be exempt from registration. However, perhaps if this industry become lucrative and more products become available, or different solutions are mixed with essential oils, the regulatory processes may change. The variation in regulatory approval processes around the world is still a barrier to the commercialisation of essential oil pesticides and hence an appropriate unanimous regulatory system needs to be established.

3.2 Conclusions

The experiments conducted as a part of this theses showed the high efficacy of 5% (v/v) concentrations of lavender and tea tree essential oils as pesticides and repellents against stable flies. Therefore, both oils are potential options as botanical alternatives to synthetic neurotoxic treatments for the control of stable flies and can be used in conjunction with the removal of material conducive to oviposition for an effective integrated pest management scheme. Furthermore, due to the broad range of ectoparasitic species affected by lavender and tea tree oil, their topical administration to animals may provide protection against a range of important pests. However, before these can be advocated for use, there must be field trials to elucidate their efficacy and residual activity under field conditions. It is likely these essential oil formulations can be incorporated into companion animal husbandry practices, but further work is needed to extend the residual activity of these oils and establish their safety before use on food production animals.

References

Achee, N.L., Sardelis, M.R., Dusfour, I., Chauhan, K.R. and Grieco, J.P. (2009) Characterization of spatial repellent, contact irritant, and toxicant chemical actions of standard vector control compounds. *Journal of the American Mosquito Control Association*, **25**, 156–167.

Adenubi, O.T., Ahmed, A.S., Fasina, F.O., McGaw, L.J., Eloff, J.N. and Naidoo, V. (2018) Pesticidal plants as a possible alternative to synthetic acaricides in tick control: A systematic review and meta-analysis. *Industrial Crops and Products*, **123**, 779–806.

Agee, H.R. and Patterson, R.S. (1983) Spectral sensitivity of stable, face, and horn flies and behavioral responses of stable flies to visual traps (Diptera: Muscidae). *Environmental Entomology,* **12**, 1823–1828.

Agrient Limited (2020) *Equine Nitnat*. [online] Available at: <https://www.agrientlimited.com/equine-nitnat> [Accessed 7 July 2020].

Aïzoun, N., Ossè, R., Azondekon, R., Alia, R., Oussou, O., Gnanguenon, V., Aikpon, R., Padonou, G.G. and Akogbéto, M. (2013) Comparison of the standard WHO susceptibility tests and the CDC bottle bioassay for the determination of insecticide susceptibility in malaria vectors and their correlation with biochemical and molecular biology assays in Benin, West Africa. *Parasites and Vectors,* **6**, 1–10.

Ajayi, O.E., Appel, A.G. and Fadamiro, H.Y. (2014) Fumigation toxicity of essential oil monoterpenes to *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae). *Journal of Insects*, **2014**, 1-7.

Anderson, J.A. and Coats, J.R. (2012) Acetylcholinesterase inhibition by nootkatone and carvacrol in arthropods. *Pesticide Biochemistry and Physiology*, **102**, 124–128.

Anderson, J.R. (1978) Mating behavior of *Stomoxys calcitrans*: effects of a blood meal on the mating drive of males and its necessity as a prerequisite for proper insemination of females. *Journal of Economic Entomology,* **71**, 379–386.

Andersson, M.N., Löfstedt, C. and Newcomb, R.D., (2015) Insect olfaction and the evolution of receptor tuning. *Frontiers in Ecology and Evolution*, **3**, 1-53.

Andress, E.R. and Campbell, J.B. (1994) Inundative releases of pteromalid parasitoids (Hymenoptera: Pteromalidae) for the control of stable flies, *Stomoxys calcitrans* (L.) (Diptera: Muscidae) at confined cattle installations in west central Nebraska. *Journal of Economic Entomology*, **87**, 714–722.

Angioni, A., Barra, A., Coroneo, V., Dessi, S. and Cabras, P. (2006) Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. stoechas essential oils from stem/leaves and Flowers. *Journal of Agricultural and Food Chemistry*, **54**, 4364–4370.

Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. (2008) Biological effects of essential oils - A review. *Food and Chemical Toxicology*, **46**, 446–475.

Baldacchino, F., Tramut, C., Salem, A., Liénard, E., Delétré, E., Franc, M., Martin, T., Duvallet, G. and Jay-Robert, P. (2013) The repellency of lemongrass oil against stable flies, tested using video tracking. *Parasite*, **20**, 1–7.

Baleba, S.B.S., Torto, B., Masiga, D., Getahun, M.N. and Weldon, C.W. (2020) Stable flies, *Stomoxys calcitrans* L. (Diptera: Muscidae), improve offspring fitness by avoiding oviposition substrates with competitors or parasites. *Frontiers in Ecology and Evolution*, **8**, 1–12.

Beadleas, M.L., Gingrich, A.R. and Miller, J.A. (1977) Slow-Release devices for livestock insect control: Cattle body surfaces contacted by five types of devices. *Journal of Economic Entomology*, **70**, 72–75.

Bedini, S., Flamini, G., Cosci, F., Ascrizzi, R., Echeverria, M.C., Gomez, E. V., Guidi, L., Landi, M., Lucchi, A. and Conti, B. (2019) Toxicity and oviposition deterrence of essential oils of *Clinopodium nubigenum* and *Lavandula angustifolia* against the myiasis-inducing blowfly *Lucilia sericata*. *PLoS ONE*, **14**, 1–17.

Bedini, S., Guarino, S., Echeverria, M.C., Flamini, G., Ascrizzi, R., Loni, A. and Conti, B. (2020) *Allium sativum, rosmarinus officinalis,* and *salvia officinalis* essential oils: A spiced shield against blowflies. *Insects*, **11**, 1–18.

Benelli, G. and Pavela, R. (2018a) Beyond mosquitoes—Essential oil toxicity and repellency against bloodsucking insects. *Industrial Crops and Products*, **117**, 382–392.

Benelli, G. and Pavela, R. (2018b) Repellence of essential oils and selected compounds against ticks—A systematic review. *Acta Tropica*, **179**, 47–54.

Bernier, U.R., Furman, K.D., Kline, D.L., Allan, S.A. and Barnard, D.R. (2005) Comparison of contact and spatial repellency of catnip oil and N,N-Diethyl-3-methylbenzamide (DEET) against mosquitoes. *Journal of Medical Entomology*, **42**, 306–311.

Berry, I. L., & Kunz, S. E. (1977) Mortality of Adult Stable Flies. *Environmental Entomology,* **6**, 569–574.

Beynon, S.A., Wainwright, W.A. and Christie, M. (2015) The application of an ecosystem services framework to estimate the economic value of dung beetles to the U.K. cattle industry. *Ecological Entomology*, **40**, 124–135.

Bischoff, K. and Guale, F. (1998) Australian tea tree (*Melaleuca alternifolia*) oil poisoning in three purebred cats. *Journal of Veterinary Diagnostic Investigation*, **10**, 208–210.

Blackwell, A., Stuart, A.E. and Estambale, B.A. (2003) The repellent and antifeedant activity of oil of *Myrica gale* against *Aedes aegypti* mosquitoes and its enhancement by the addition of

salicyluric acid. *The Journal of the Royal College of Physicians of Edinburgh,* **33**, 209-214.

Bohbot, J.D. and Dickens, J.C. (2010) Insect repellents: modulators of mosquito odorant receptor activity. *PLoS One*, **5,** 1-11

Bouzeraa, H., Bessila-Bouzeraa, M. and Labed, N., (2019) Repellent and fumigant toxic potential of three essential oils against *Ephestia kuehniella*. *Biosystems Diversity*, **27**, 349-353.

Braverman, Y., Ungar-Waron, H., Frith, K., Adler, H., Danieli, Y., Baker, K. and Quinn, P. (1983) Epidemiological and immunological studies of sweet itch in horses in Israel. *Veterinary Record,* **112**, 512–524.

Burgess, I.F. (2009) The mode of action of dimeticone 4% lotion against head lice, *Pediculus capitis. BioMed Central Pharmacology*, **9**, 1–8.

Burkhard, P.R., Burkhardt, K., Landis, T. and Haenggeli, C.A. (1999) Plant-induced seizures: Reappearance of an old problem. *Journal of Neurology*, **246**, 667–670.

Buschman, L.L. and Patterson, R.S. (1981) Assembly, mating, and thermoregulating behavior of stable flies under field conditions. *Environmental Entomology*, **10**, 16–21.

Butler, J.F., Kloft, W.J., DuBose, L.A. and Kloft, E.S. (1977) Recontamination of food after feeding a 32P food source to biting Muscidae. *Journal of Medical Entomology*, **13**, 567-571.

Callander, J.T. and James, P.J. (2012) Insecticidal and repellent effects of tea tree (*Melaleuca alternifolia*) oil against *Lucilia cuprina*. *Veterinary Parasitology*, **184**, 271–278.

Campbell, J.B. (1977) Horn, face and stable fly control with feed additive insecticides. *Insecticide and Acaricide Tests*, **2**, 136–137.

Campbell, J.B., Skoda, S.R., Berkebile, D.R., Boxler, D.J., Thomas, G.D., Adams, D.C. and Davis, R. (2001) Effects of stable flies (Diptera: Muscidae) on weight gains of grazing yearling cattle. *Journal of Economic Entomology*, **94**, 780–783.

Campbell, W.C. (1985) Ivermectin: An update. *Parasitology Today,* **1**, 10–16.

Cantalamessa, F. (1993) Acute toxicity of two pyrethroids, permethrin, and cypermethrin in neonatal and adult rats. *Archives of Toxicology*, **67**, 510-513.

Cárdenas, J., Rojas, J., Rondón, M. and Nieves, E. (2012) Adulticide effect of *Monticalia greenmaniana* (Asteraceae) against *Lutzomyia migonei* (Diptera: Psychodidae). *Parasitology Research,* **111**, 787–794.

Cavanagh, H.M.A. and Wilkinson, J.M. (2002) Biological activities of lavender essential oil. *Phytotherapy Research*, **16,** 301-308.

Centers for Disease Control and Prevention (2011) Guideline for Evaluating Insecticide Resistance in Vectors Using the CDC Bottle Bioassay. *Centers for Disease Control and Prevention,* 11-14.

Chaaban, A., Gomes, E.N., Richardi, V.S., Martins, C.E.N., Brum, J.S., Navarro-Silva, M.A., Deschamps,

C. and Molento, M.B. (2019b) Essential oil from *Curcuma longa* leaves: Can an overlooked by-product from turmeric industry be effective for myiasis control? *Industrial Crops and Products*, **132**, 352–364.

Chaaban, A., Martins, C.E.N., Bretanha, L.C., Micke, G.A., Carrer, A.R., Rosa, N.F., Ferreira, L. and Molento, M.B. (2018a) Insecticide activity of *Baccharis dracunculifolia* essential oil against *Cochliomyia macellaria* (Diptera: Calliphoridae*). Natural Product Research*. **32**, 2954–2958.

Chaaban, A., Richardi, V.S., Carrer, A.R., Brum, J.S., Cipriano, R.R., Martins, C.E.N., Silva, M.A.N., Deschamps, C. and Molento, M.B. (2019a) Insecticide activity of *Curcuma longa* (leaves) essential oil and its major compound α-phellandrene against *Lucilia cuprina* larvae (Diptera: Calliphoridae): Histological and ultrastructural biomarkers assessment. *Pesticide Biochemistry and Physiology*, **153**, 17–27.

Chaaban, A., Santos, V.M.C.S., Gomes, E.N., Martins, C.E.N., do Amaral, W., Deschamps, C. and Molento, M.B. (2018b) Chemical composition of *piper gaudichaudianum* essential oil and its bioactivity against *Lucilia cuprina* (Diptera: Calliphoridae). *Journal of Essential Oil Research*, **30**, 159–166.

Chil-Núñez, I., Mendonça, P.M., Escalona-Arranz, J.C., Cortinhas, L.B., Dutok-Sánchez, C.M. and de Carvalho Queiroz, M.M. (2018) Insecticidal effects of *Ocimum sanctum* var. cubensis essential oil on the diseases vector *Chrysomya putoria*. *Journal of Pharmacy and Pharmacognosy Research,* **6**, 148–157.

Christen, V., Joho, Y., Vogel, M. and Fent, K. (2019) Transcriptional and physiological effects of the pyrethroid deltamethrin and the organophosphate dimethoate in the brain of honeybees (*Apis mellifera*). *Environmental Pollution*, **244**, 247–256.

Cilek, J.E. (1999) Evaluation of various substances to increase adult *Stomoxys calcitrans* (Diptera: Muscidae) collections on alsynite cylinder traps in North Florida. *Journal of Medical Entomology*, **36**, 605–609.

Cilek, J.E. and Greene, G.L. (1994) Stable fly (Diptera: Muscidae) insecticide resistance in Kansas cattle feedlots. *Journal of Economic Entomology*, **87**, 275–279.

Colwell, D.D., Kavaliers, M. and Lysk, T. (1997) Stable fly, *Stomoxys calcitrans*, mouthpart removal influences stress and anticipatory responses in mice. *Medical and Veterinary Entomology*, **11**, 310–314.*Medical and Veterinary Entomology*, **11**, 310–314.

Corcos, D., Mazzon, L., Cerretti, P., Mei, M., Giussani, E., Drago, A. and Marini, L. (2019) Effects of natural pyrethrum and synthetic pyrethroids on the tiger mosquito, *Aedes albopictus* (skuse) and non-target flower-visiting insects in urban green areas of Padua, Italy. *International Journal of Pest Management*, **66**, 215–221.

Cossetin, L.F., Santi, E.M.T., Cossetin, J.F., Dillmann, J.B., Baldissera, M.D., Garlet, Q.I., De Souza, T.P., Loebens, L., Heinzmann, B.M., Machado, M.M. and Monteiro, S.G. (2018) In vitro safety and efficacy of lavender essential oil (Lamiales: Lamiaceae) as an insecticide against houseflies (Diptera: Muscidae) and blowflies (Diptera: Calliphoridae). *Journal of Economic Entomology*, **111**, 1974–1982.

Costa-Júnior, L.M., Miller, R.J., Alves, P.B., Blank, A.F., Li, A.Y. and Pérez de León, A.A. (2016) Acaricidal efficacies of *Lippia gracilis* essential oil and its phytochemicals against organophosphate-resistant and susceptible strains of *Rhipicephalus (Boophilus) microplus*. *Veterinary Parasitology*, **228**, 60–64.

Costa, C.A.R. de A., Kohn, D.O., De Lima, V.M., Gargano, A.C., Flório, J.C. and Costa, M. (2011) The GABAergic system contributes to the anxiolytic-like effect of essential oil from *Cymbopogon citratus* (lemongrass). *Journal of Ethnopharmacology*, **137**, 828–836.

Cox, S.D., Mann, C.M., Markham, J.L., Bell, H.C., Gustafson, J.E., Warmington, J.R. and Wyllie, S.G. (2000) The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *Journal of Applied Microbiology*, **88**, 170-175.

Cox, S.D., Mann, C.M., Markham, J.L., Gustafson, J.E., Warmington, J.R. and Wyllie, S.G. (2001) Determining the antimicrobial actions of tea tree oil. *Molecules*, **6**, 87-91.

Cumming, J. (1998) Diptera associated with livestock dung. [online] *North America Dipterists Society*. Available at: <http://www.nadsdiptera.org/FFP/stable.htm> [Accessed 9 April 2020].

Cutkomp, L.K. and Harvey, A.L. (1958) The weight responses of beef cattle in relation to control of horn and stable flies. *Journal of Economic Entomology*, **51**, 72–75.

de Oliveira, B.M.S., Melo, C.R., Alves, P.B., Santos, A.A., Santos, A.C.C., Santana, A.D.S., Araújo, A.P.A., Nascimento, P.E.S., Blank, A.F. and Bacci, L. (2017) Essential oil of aristolochia trilobata: Synthesis, routes of exposure, acute toxicity, binary mixtures and behavioral effects on leaf-cutting ants. *Molecules*, **22**, 1-17.

Dougherty, C.T., Knapp, F.W., Burrus, P.B., Willis, D.C. and Cornelius, P.L. (1994) Moderation of grazing behavior of beef cattle by stable flies (*Stomoxys calcitrans* L.). *Applied Animal Behaviour Science*, **40**, 113–127.

Dougherty, C.T., Knapp, F.W., Burrus, P.B., Willis, D.C., Cornelius, P.L. and Bradley, N.W. (1993) Multiple releases of stable flies (*Stomoxys calcitrans* L.) and behaviour of grazing beef cattle. *Applied Animal Behaviour Science*, **38**, 191-212.

Dsouli, N., Delsuc, F., Michaux, J., De Stordeur, E., Couloux, A., Veuille, M. and Duvallet, G. (2011) Phylogenetic analyses of mitochondrial and nuclear data in haematophagous flies support the paraphyly of the genus *Stomoxys* (Diptera: Muscidae). *Infection, Genetics and Evolution*, **11**,

663-670.

El Ashmawy, W.R., Williams, D.R., Gerry, A.C., Champagne, J.D., Lehenbauer, T.W. and Aly, S.S. (2019) Risk factors affecting dairy cattle protective grouping behavior, commonly known as bunching, against *Stomoxys calcitrans* (L.) on California dairies. *PLoS ONE*, **14**, 1–22.

Ellse, L. and Wall, R. (2014) The use of essential oils in veterinary ectoparasite control: A review. *Medical and Veterinary Entomology*, **28**, 233–243.

Ellse, L., Sands, B., Burden, F.A. and Wall, R. (2016) Essential oils in the management of the donkey louse, *Bovicola ocellatus. Equine Veterinary Journal*, **48**, 285–289.

Enan, E. (2001) Insecticidal activity of essential oils: Octopaminergic sites of action. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology,* **130**, 325–337.

Enan, E.E. (2005) Molecular and pharmacological analysis of an octopamine receptor from American cockroach and fruit fly in response to plant essential oils. *Archives of Insect Biochemistry and Physiology,* **59**, 161–171.

Farhat, G.N., Affara, N.I. and Gali-Muhtasib, H.U. (2001) Seasonal changes in the composition of the essential oil extract of East Mediterranean sage (*Salvia libanotica*) and its toxicity in mice. *Toxicon*, **39**, 1601–1605.

Farnsworth, W.R., Collett, M.G. and Ridley, I.S. (1997) Field Survey of Insecticide Resistance in *Haematobia irritans* exigua de Meijere (Diptera: Muscidae). *Australian Journal of Entomology*, **36**, 257–261.

Feaster, J.E., Scialdone, M.A., Todd, R.G., Gonzalez, Y.I., Foster, J.P. and Hallahan, D.L. (2009) Dihydronepetalactones deter feeding activity by mosquitoes, stable flies, and deer ticks. *Journal of Medical Entomology*, **46**, 832–840.

Ferreira, T.P., Haddi, K., Corrêa, R.F.T., Zapata, V.L.B., Piau, T.B., Souza, L.F.N., Santos, S.M.G., Oliveira, E.E., Jumbo, L.O.V., Ribeiro, B.M., Grisolia, C.K., Fidelis, R.R., Maia, A.M.S. and Aguiar, R.W.S. (2019) Prolonged mosquitocidal activity of *Siparuna guianensis* essential oil encapsulated in chitosan nanoparticles. *PLoS Neglected Tropical Diseases*, **13**, 1–23.

Florez-Cuadros, M., Berkebile, D., Brewer, G. and Taylor, D.B. (2019) Effects of diet quality and temperature on stable fly (Diptera: Muscidae) development. *Insects*, **10**, 1–13.

Foil, L.D. and Hogsette, J.A. (1994) Biology and control of tabanids, stable flies and horn flies. *Revue Scientifique et Technique*, **13**, 1125–1158.

Food and Agriculture Organization of the United Nations (2004) Food and Agriculture Organization of the United Nations, Module 1. Ticks: acaricide resistance: diagnosis management and prevention. In: *Guidelines Resistance Management and Integrated Parasite Control In Ruminants*, FAO Animal Production and Health Division, Rome, Italy.

Fouche, G., Adenubi, O.T., Leboho, T., McGaw, L.J., Naidoo, V., Wellington, K.W. and Eloff, J.N. (2019) Acaricidal activity of the aqueous and hydroethanolic extracts of 15 South African plants against *Rhipicephalus turanicus* and their toxicity on human liver and kidney cells. *Onderstepoort Journal of Veterinary Research*, **86**, 1–7.

Fouche, G., Sakong, B.M., Adenubi, O.T., Dzoyem, J.P., Naidoo, V., Leboho, T., Wellington, K.W. and Eloff, J.N. (2017) Investigation of the acaricidal activity of the acetone and ethanol extracts of 12 South African plants against the adult ticks of *Rhipicephalus turanicus*. *Onderstepoort Journal of Veterinary Research*, **84**, 1–6.

Galli, G.M., Roza, L.F., Santos, R.C., Quatrin, P.M., Ourique, A.F., Klein, B., Wagner, R., Baldissera, M.D., Volpato, A., Campigotto, G. and Glombowsky, P. (2018) Low dose of nanocapsules containing eucalyptus oil has beneficial repellent effect against horn fly (Diptera: Muscidae). *Journal of Economic Entomology*, **111**, 2983-2987.

Gebremichael, S., Birhanu, T. and Tessema, D.A. (2013) Analysis of organochlorine pesticide residues in human and cow's milk in the towns of Asendabo, Serbo and Jimma in South-Western Ethiopia. *Chemosphere*, **90**, 1652–1657.

George, D.R., Callaghan, K., Guy, J.H. and Sparagano, O.A. (2008) Lack of prolonged activity of lavender essential oils as acaricides against the poultry red mite (*Dermanyssus gallinae*) under laboratory conditions. *Research in Veterinary Science*, **85**, 540–542.

Gilles, J., David, J.-F. and Duvallet, G. (2005) Temperature effects on development and survival of two stable flies, *Stomoxys calcitrans* and *Stomoxys niger niger* (Diptera: Muscidae), in La Réunion Island. *Journal of Medical Entomology*, **42**, 260–265.

González, M., Venter, G.J., López, S., Iturrondobeitia, J.C. and Goldarazena, A. (2014) Laboratory and field evaluations of chemical and plant-derived potential repellents against Culicoides biting midges in northern Spain. *Medical and Veterinary Entomology*, **28**, 421–431.

Goode, P., Ellse, L. and Wall, R. (2018) Preventing tick attachment to dogs using essential oils. *Ticks and Tick-borne Diseases*, **9,** 921-926.

Greive, K.A., Staton, J.., Miller, P.F., Peters, B.A. and Oppenheim, V.M.J. (2010) Development of *Melaleuca* oils as effective natural-based personal insect repellents. *Australian Journal of Entomology*, **49**, 40–48.

Hagvall, L., Sköld, M., Bråred-Christensson, J., Börje, A. and Karlberg, A.T. (2008) Lavender oil lacks natural protection against autoxidation, forming strong contact allergens on air exposure. *Contact Dermatitis*, **59**, 143–150.

Hammer, K., Carson, C.F. and Riley, T.V. (2003) Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. *Journal of Applied Microbiology*, **95**, 853-860.

Harris, R.L., Miller, J.A. and Frazar, E.D. (1974) Horn flies and stable flies: Feeding activity. *Annals of the Entomological Society of America*, **67,** 891-894.

Hazarika, S., Dhiman, S., Rabha, B., Bhola, R. and Singh, L. (2012) Repellent activity of some essential oils against *Simulium* species in India. *Journal of Insect Science*, **12**, 1–9.

Herath, H.M.W., Iruthayathas, E.E. and Orod, D.P. (1979) Temperature effects on essential oil composition of citronella selections. *Economic Botany,* **33**, 425–430.

Herholz, C., Kopp, C., Wenger, M., Mathis, A., Wägeli, S. and Roth, N. (2016) Efficacy of the repellent N, N-diethyl-3-methyl-benzamide (DEET) against tabanid flies on horses evaluated in a field test in Switzerland. *Veterinary Parasitology*, **221**, 64-67.

Herman, A. and Herman, A.P. (2015) Essential oils and their constituents as skin penetration enhancer for transdermal drug delivery: a review. *Journal of Pharmacy and Pharmacology*, **67**, 473-485.

Hieu, T.T., Jung, J., Kim, S.I., Ahn, Y.-J. and Kwon, H.W. (2014) Behavioural and electroantennogram responses of the stable fly (*Stomoxys calcitrans* L.) to plant essential oils and their mixtures with attractants. *Pest Management Science*, **70**, 163–172.

Hieu, T.T., Kim S.I., Kwon, H.W., and Ahn, Y.S. (2010a) Enhanced repellency of binary mixtures of *Zanthoxylum piperitum* pericarp steam distillate or *Zanthoxylum armatum* seed oil constituents and *Calophyllum inophyllum* nut oil and their aerosols to *Stomoxys calcitrans*. *Pest Management Science*, **66**, 1191–1198.

Hieu, T.T., Kim, S.I., Lee, S.-G. and Ahn, Y.J. (2010b) Repellency to *Stomoxys calcitrans* (Diptera: Muscidae) of plant essential oils alone or in combination with *Calophyllum inophyllum* nut oil. *Journal of Medical Entomology*, **47**, 575–580.

Hogsette, J. A and J. P. Ruff. (1986) Evaluation of flucythrinate- and fenvalerate-impregnated ear tags and permethrin ear tapes for fly control on beef and dairy cattle in northwest Florida. *Journal of Economic Entomology*, **79**, 152-157.

Hogsette, J.A. (1983) An attractant self-marking device for marking field populations of stable flies with fluorescent dusts. *Journal of Economic Entomology*, **76**, 510-514.

Hogsette, J.A. and Kline, D.L. (2017) The Knight Stick Trap and Knight Stick Sticky Wraps: New tools for stable fly (Diptera: Muscidae) management. *Journal of Economic Entomology*, **110**, 1384-1389

Hogsette, J.A. and Ose, G.A. (2017) Improved capture of stable flies (Diptera: Muscidae) by placement of knight stick sticky fly traps protected by electric fence inside animal exhibit yards at the Smithsonian's National Zoological Park. *Zoo Biology*, **36**, 382–386.

Hogsette, J.A. and Ruff, J.P. (1985) Stable fly (Diptera: Muscidae) migration in northwest

Florida. *Environmental Entomology*, **14**, 170-175.

Hogsette, J.A., Ruff, J.P. and Jones, C.J. (1987) Stable fly biology and control in northwest Florida. *Journal of Agricultural Entomology*, **4**, 1–11.

Holdsworth, P.A., Vercruysse, J., Rehbein, S., Peter, R.J., De Bruin, C., Letonja, T. and Green, P. (2006) World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of ectoparasiticides against biting and nuisance flies on ruminants. *Veterinary Parasitology*, **136**, 3-13.

Hollingworth, R.M., Ahammadsahib, K.I., Gadelhak, G. and McLaughlin, J.L. (1994) New inhibitors of complex I of the mitochondrial electron transport chain with activity as pesticides. *Biochemical Society Transactions*, **22**, 230-233.

Holm, Y., Laakso, I., Hiltunen, R. and Galambosi, B. (1997) Variation in the essential oil composition of *Artemisia annua* L. of different origin cultivated in Finland. *Flavour and Fragrance Journal*, **12**, 241–246.

International Organization for Standardization (IOS), 2017. *Essential oil of Melaleuca, terpinen-4-ol type (tea tree oil)*. [online] Geneva: International Organization for Standardization. Available at: <https://www.sis.se/api/document/preview/921469> [Accessed 22 March 2020].

Isman, M. B. (1997) Neem and other Botanical insecticides: Barriers to commercialisation. *Phytoparasitica,* **25**, 339.

Isman, M.B. (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review Entomology*, **51**, 45–66.

Isman, M.B. (2017) Bridging the gap: moving botanical insecticides from the laboratory to the farm. *Industrial Crops and Products*, **110**, 10-14.

Isman, M.B. (2019) Commercial development of plant essential oils and their constituents as active ingredients in bioinsecticides. *Phytochemistry Reviews*, **19**, 235–241.

Isman, M.B. and Grieneisen, M.L. (2014) Botanical insecticide research: Many publications, limited useful data. *Trends in Plant Science*, **19**, 140–145.

James, P.J. and Callander, J.T. (2012) Dipping and jetting with tea tree (*Melaleuca alternifolia*) oil formulations control lice (*Bovicola ovis*) on sheep. *Veterinary Parasitology*, **189**, 338-343.

Jankowska, M., Rogalska, J., Wyszkowska, J. and Stankiewicz, M. (2018) Molecular targets for components of essential oils in the insect nervous system—a review. *Molecules*, **23**, 1–20.

Juan, L.W., Lucia, A., Zerba, E.N., Harrand, L., Marco, M. and Masuh, H.M. (2011) Chemical composition and fumigant toxicity of the essential oils from 16 species of Eucalyptus against *Haematobia irritans* (Diptera: Muscidae) adults. *Journal of Economic Entomology*, **104**, 1087-1092.

Karasek, I., Butler, C., Baynes, R. and Werners, A. (2020) A review on the treatment and control of ectoparasite infestations in equids. *Journal of Veterinary Pharmacology and Therapeutics*, **0**, 1-8.

Khater, H.F. (2014) Bioactivities of some essential oils against the camel nasal botfly, *Cephalopina titillator. Parasitology Research*, **113,** 593–605.

Khater, H.F. and Geden, C.J. (2018) Potential of essential oils to prevent fly strike and their effects on the longevity of adult *Lucilia sericata. Journal of Vector Ecology,* **43**, 261–270.

Khater, H.F., Hanafy, A., Abdel-Mageed, A.D., Ramadan, M.Y. and El-Madawy, R.S. (2011) Control of the myiasis-producing fly, *Lucilia sericata*, with Egyptian essential oils. *International Journal of Dermatology,* **50**, 187–194.

Khater, H.F., Ramadan, M.Y. and El-Madawy, R.S. (2009) Lousicidal, ovicidal and repellent efficacy of some essential oils against lice and flies infesting water buffaloes in Egypt. *Veterinary Parasitology*, **164**, 257–266.

Killough, R. A., and Mckinstry, D. M. (1965) Mating and Oviposition Studies of the Stable Fly. *Journal of Economic Entomology*, **58**, 489–491.

Kim, S.I., Yoon, J.S., Baeck, S.J., Lee, S.H., Ahn, Y.J. and Kwon, H.W. (2012) Toxicity and synergic repellency of plant essential oil mixtures with vanillin against *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, **49,** 876-885.

Klauck, V., Pazinato, R., Radavelli, W.M., Volpato, A., Stefani, L.M. and da Silva, A.S. (2015) *In vitro* repellent effect of tea tree (*Melaleuca alternifolia*) and andiroba (*Carapa guianensis*) oils on *Haematobia irritans* and *Chrysomya megacephala* flies. *Tropical Biomedicine*, **32**, 33-39.

Klauck, V., Pazinato, R., Stefani, L.M., Santos, R.C., Vaucher, R.A., Baldissera, M.D., Raffin, R., Boligon, A., Athayde, M., Baretta, D. and Machado, G. (2014) Insecticidal and repellent effects of tea tree and andiroba oils on flies associated with livestock. *Medical and Veterinary Entomology*, **28**, 33-39.

Kohari, D., Hongo, T. and Inoue, K. (2020) The influence of stable fly invasion on the behavior of captive black rhinoceros (*Diceros bicornis*). *Journal of Veterinary Behavior,* **35**, 83–87.

Kosgei, C.J., Matasyoh, J.C., Mwendia, C.M., Kariuki, S.T. and Guliye, A.Y. (2014) Chemical composition and larvicidal activity of essential oil of *Lippia kituiensis* against larvae of *Rhipicephalus appendiculatus. International Journal of Biological and Chemical Sciences,* **8**, 1938–1947.

Koutsaviti, A., Antonopoulou, V., Vlassi, A., Antonatos, S., Michaelakis, A., Papachristos, D.P. and Tzakou, O. (2018) Chemical composition and fumigant activity of essential oils from six plant families against *Sitophilus oryzae* (Col: Curculionidae). *Journal of Pest Science*, **91**, 873–886.

Lachance, S. and Grange, G. (2014) Repellent effectiveness of seven plant essential oils, sunflower oil and natural insecticides against horn flies on pastured dairy cows and heifers. *Medical and Veterinary Entomology*, **28**, 193–200.

Lange, B.M., Mahmoud, S.S., Wildung, M.R., Turner, G.W., Davis, E.M., Lange, I., Baker, R.C., Boydston, R.A. and Croteau, R.B. (2011) Improving peppermint essential oil yield and composition by metabolic engineering. *Proceedings of the National Academy of Sciences*, **108**, 16944-16949

López, M.D. and Pascual-Villalobos, M.J. (2010) Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. *Industrial Crops and Products*, **31**, 284–288.

López, M.D., Campoy, F.J., Pascual-Villalobos, M.J., Muñoz-Delgado, E. and Vidal, C.J. (2015) Acetylcholinesterase activity of electric eel is increased or decreased by selected monoterpenoids and phenylpropanoids in a concentration-dependent manner. *Chemico-Biological Interactions*, **229**, 36–43

Lwande, W., Ndakala, A.J., Hassanali, A., Moreka, L., Nyandat, E., Ndungu, M., Amiani, H., Gitu, P.M., Malonza, M.M. and Punyua, D.K. (1999) *Gynandropsis gynandra* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) repellents. *PhytoChemistry*, **50**, 401–405.

Lysk, T. (1993) Seasonal abundance of stable flies and house flies (Diptera:Muscidae) in dairies in Alberta, Canada. *Journal of Medical Entomology*, **30**, 888–895.

Machtinger, E.T., Leppla, N.C. and Hogsette, J.A. (2016) House and stable fly seasonal abundance, larval development substrates, and natural parasitism on small equine farms in Florida. *Neotropical Entomology,* **45**, 433–440.

Maciel, M. V., Morais, S.M., Bevilaqua, C.M.L., Silva, R.A., Barros, R.S., Sousa, R.N., Sousa, L.C., Brito, E.S. and Souza-Neto, M.A. (2010) Chemical composition of *Eucalyptus* spp. essential oils and their insecticidal effects on *Lutzomyia longipalpis*. *Veterinary Parasitology,* **167**, 1–7.

Maes, C., Bouquillon, S. and Fauconnier, M.L. (2019) Encapsulation of essential oils for the development of biosourced pesticides with controlled release: A review. *Molecules*, **24**, 1–17.

Mägi, E., Järvis, T. and Miller, I. (2006) Effects of different plant products against pig mange mites. *Acta Veterinaria Brno*, **75**, 283-287.

Mahmoud, S.S. and Croteau, R.B. (2001) Metabolic engineering of essential oil yield and composition in mint by altering expression of deoxyxylulose phosphate reductoisomerase and menthofuran synthase. *Proceedings of the National Academy of Sciences*, **98**, 8915-8920.

Markus Lange, B. and Turner, G.W. (2013) Terpenoid biosynthesis in trichomes—current status and future opportunities. *Plant Biotechnology Journal*, **11***,* 2-22.

Masmeatathip, R., Ketavan, C. and Duvallet, G. (2006) Morphological studies of *Stomoxys* spp.

(Diptera: Muscidae) in central Thailand. *Agriculture and Natural Resources*, **40**, 872-881.

Masotti, V., Juteau, F., Bessière, J.M. and Viano, J. (2003) Seasonal and phenological variations of the essential oil from the narrow endemic species *Artemisia molinieri* and its biological activities. *Journal of Agricultural and Food Chemistry*, **51**, 7115–7121.

Mekonnen, A., Tesfaye, S., Christos, S.G., Dires, K., Zenebe, T., Zegeye, N., Shiferaw, Y. and Lulekal, E. (2019) Evaluation of skin irritation and acute and subacute oral toxicity of *Lavandula angustifolia* essential oils in rabbit and mice. *Journal of Toxicology*, **2019***,* 1-8.

Mellor, P.S., Kitching, R.P. and Wilkinson, P.J. (1987) Mechanical transmission of capripox virus and African swine fever virus by *Stomoxys calcitrans*. *Research in Veterinary Science*, **43**, 109–112.

Meyer, J.A. and Petersen, J.J. (1983) Characterization and seasonal distribution of breeding sites of stable flies and house flies (Diptera: Muscidae) on eastern Nebraska feedlots and dairies. *Journal of Economic Entomology*, **76**, 103-108.

Miller, R.W., Pickens, L.G., Morgan, N.O., Thimlian, R.W. and Wilson, R.L. (1973) Effect of stable flies on feed intake and milk production of dairy cows. *Journal of Economic Entomology,* **66**, 711–714.

Mills, C., Cleary, B. V., Walsh, J.J. and Gilmer, J.F. (2004) Inhibition of acetylcholinesterase by Tea Tree oil. *Journal of Pharmacy and Pharmacology*, **56**, 375–379.

Mills, P.C. (2007) Vehicle effects on the in vitro penetration of testosterone through equine skin. *Veterinary Research Communications*, **31***,* 227-233.

Mills, P.C., Magnusson, B.M. and Cross, S.E. (2005) Effects of vehicle and region of application on absorption of hydrocortisone through canine skin. *American Journal of Veterinary Research*, **66**, 43-47.

Misharina, T.A., Polshkov, A.N., Ruchkina, E.L. and Medvedeva, I.B. (2003) Changes in the composition of the essential oil in stored marjoram. *Applied Biochemistry and Microbiology*, **39**, 353–358.

Mkolo, M.N. and Magano, S.R. (2007) Repellent effects of the essential oil of *Lavendula angustifolia* against adults of *Hyalomma marginatum rufipes*. *Journal of the South African Veterinary Association*, **78**, 149–152.

Morgan, D., Bailie, H. (1980) A field trial to determine the effect of fly control using permethrin on milk yields in dairy cattle in the UK. *Veterinary Record*, **106**, 121-123.

Morrison, P.E., Venkatesh, K. and Thompson, B. (1982) The role of male accessory-gland substance on female reproduction with some observations of spermatogenesis in the stable fly. *Journal of Insect Physiology*, **28**, 607–614.

Mossa, A.H., Mohafrash, S.M.M. and Chandrasekaran, N. (2018) Safety of natural insecticides: Toxic

effects on experimental animals. *BioMed Research International,* **2018**, 1–17.

Mottet, R.S., Moon, R.D., Hathaway, M.R. and Martinson, K.L. (2018) Effectiveness of Stable Fly Protectants on Adult Horses. *Journal of Equine Veterinary Science*, **69**, 11–15.

Moyo, B. and Masika, P.J. (2013) Validation of the acaricidal properties of materials used in ethno-veterinary control of cattle ticks. *African Journal of Microbiology Research*, **7**, 4701–4706.

Mullens, B.A., Watson, D.W., Gerry, A.C., Sandelin, B.A., Soto, D., Rawls, D., Denning, S., Guisewite, L. and Cammack, J. (2017) Field trials of fatty acids and geraniol applied to cattle for suppression of horn flies, *Haematobia irritans* (Diptera: Muscidae), with observations on fly defensive behaviors. *Veterinary Parasitology*, **245**, 14–28.

Munoz-Bertomeu, J., Arrillaga, I., Ros, R. and Segura, J. (2006) Metabolic engineering of essential oil yield in spike lavender. *Plant Physiology*, **142***,* 890-900.

Muraleedharan, K. (2005) Pyrethroid insecticides, Sumicidin and Butox for the effective control of *Stomoxys calcitrans. Journal of Parasitic Diseases*, **29**, 164–165.

NAF UK (2020) *NAF Off Citronella*. [online] Available at: <https://www.naf-equine.eu/uk/care/naf-off-citronella> [Accessed 8 July 2020].

Naissance. 2020. *Ethically sourced natural oils and ingredients, Naissance*. [online] Available at: <https://naissance.com/> [Accessed 18 April 2020].

Najafian, S. (2016) The effect of time and temperature on the shelf life of essential oils of Lavandula officinalis. *Journal of Essential Oil Research*, **28**, 413–420.

Nchu, F., Magano, S.R. and Eloff, J.N. (2012) In vitro anti-tick properties of the essential oil of *Tagetes minuta* L. (Asteraceae) on *Hyalomma rufipes* (Acari: Ixodidae). *Onderstepoort Journal of Veterinary Research*, **79**, 1–5.

Ndungu, M., Lwande, W., Hassanali, A., Moreka, L. and Chhabra, S.C. (1995) *Cleome monophylla* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) and maize weevil (*Sitophilus zeamais*) repellents. *Entomologia Experimentalis et Applicata*, **76**, 217–222.

Nerio, L.S., Olivero-Verbel, J. and Stashenko, E. (2010) Repellent activity of essential oils: A review. *Bioresource Technology*, **101**, 372–378.

Nieves, E., Méndez, J.F., Lias, J., Rondón, M. and Briceño, B. (2010) Actividad repelente de aceites esenciales contra las picaduras de *Lutzomyia migonei* (Diptera: Psychodidae). *Revista de Biologia Tropical,* **58**, 1549–1560.

Odeniran, P.O., Macleod, E.T., Ademola, I.O. and Welburn, S.C. (2019) Molecular identification of bloodmeal sources and trypanosomes in *Glossina* spp., *Tabanus* spp. and *Stomoxys* spp. trapped on cattle farm settlements in southwest Nigeria. *Medical and Veterinary Entomology*, **33**, 269–281.

Orchard, I. (1982) Octopamine in insects: neurotransmitter, neurohormone, and neuromodulator. *Canadian Journal of Zoology*, **60**, 659–669.

Ose, G.A. and Hogsette, J.A. (2014) Spatial distribution, seasonality and trap preference of stable fly, *Stomoxys calcitrans* L. (Diptera: Muscidae), adults on a 12-hectare zoological park. *Zoo Biology*, **33**, 228–233.

Oyedele, A. O., Gbolade, A. A., Sosan, M. B., Adewovin, F. B., Soyelu, O. L. and Orafidiva, O. O. (2002) Formulation of an effective mosquito-repellent topical product from lemongrass oil. *Phytomedicine,* **9**, 259-262.

Papachristos, D.P., Karamanoli, K.I., Stamopoulos, D.C. and Menkissoglu-Spiroudi, U. (2004) The relationship between the chemical composition of three essential oils and their insecticidal activity against *Acanthoscelides obtectus* (Say). *Pest Management Science: formerly Pesticide Science*, ***60***, 514-520.

Park, C.G., Jang, M., Yoon, K.A. and Kim, J. (2016) Insecticidal and acetylcholinesterase inhibitory activities of Lamiaceae plant essential oils and their major components against *Drosophila suzukii* (Diptera: Drosophilidae). *Industrial Crops and Products*, **89**, 507–513.

Park, I.K. (2014) Fumigant toxicity of oriental sweetgum (*Liquidambar orientalis*) and valerian (*Valeriana wallichii*) essential oils and their components, including their acetylcholinesterase inhibitory activity, against Japanese termites (*Reticulitermes speratus*). *Molecules*, **19**, 12547–12558.

Parravani, A., Chivers, C.A., Bell, N., Long, S., Burden, F. and Wall, R. (2019) Seasonal abundance of the stable fly *Stomoxys calcitrans* in southwest England. *Medical and Veterinary Entomology*, **33**, 485–490.

Patra, G., Behera, P., Das, S., Saikia, B., Ghosh, S., Biswas, P., Kumar, A., Alam, S., Kawlni, L., Lalnunpuia, C., Lalchhandama, C., Bachan, M. and Debbarma, A. (2018) *Stomoxys calcitrans* and its importance in livestock : a review. *International Journal of Advance Agricultural Research*, **6**, 30–37.

Pavela, R. (2014) Insecticidal properties of *Pimpinella anisum* essential oils against the *Culex quinquefasciatus* and the non-target organism *Daphnia magna*. *Journal of Asia-Pacific Entomology*, **17**, 287–293.

Pavela, R. and Govindarajan, M. (2016) The essential oil from *Zanthoxylum monophyllum* a potential mosquito larvicide with low toxicity to the non-target fish *Gambusia affinis*. *Journal of Pest Science*, **90**, 369–378.

Pavela, R. and Sedlák, P. (2018) Post-application temperature as a factor influencing the insecticidal activity of essential oil from *Thymus vulgaris*. *Industrial Crops and Products*, **113**, 46–49.

Pazinato, R., Klauck, V., Volpato, A., Tonin, A.A., Santos, R.C., de Souza, M.E., Vaucher, R.A., Raffin, R., Gomes, P., Felippi, C.C. and Stefani, L.M. (2014) Influence of tea tree oil (*Melaleuca alternifolia*) on the cattle tick *Rhipicephalus microplus*. *Experimental and Applied Acarology*, **63**, 77-83.

Périno-Issartier, S., Ginies, C., Cravotto, G. and Chemat, F. (2013) A comparison of essential oils obtained from lavandin via different extraction processes: Ultrasound, microwave, turbohydrodistillation, steam and hydrodistillation. *Journal of Chromatography A*, **1305**, 41-47.

Périno-Issartier, S., Ginies, C., Cravotto, G. and Chemat, F. (2013) A comparison of essential oils obtained from lavandin via different extraction processes: Ultrasound, microwave, turbohydrodistillation, steam and hydrodistillation. *Journal of Chromatography A*, **1305**, 41-47.

Perry, N.B., Anderson, R.E., Brennan, N.J., Douglas, M.H., Heaney, A.J., McGimpsey, J.A. and Smallfield, B.M. (1999) Essential oils from Dalmatian sage (*Salvia officinalis* L.): Variations among individuals, plant parts, seasons, and sites. *Journal of Agricultural and Food Chemistry*, **47**, 2048–2054.

Peterson, C.J., Nemetz, L.T., Jones, L.M. and Coats, J.R. (2002. Behavioral activity of catnip (Lamiaceae) essential oil components to the German cockroach (Blattodea: Blattellidae). *Journal of Economic Entomology*, **95**, 377–380.

Pitzer, J.B., Kaufman, P.E. and Tenbroeck, S.H. (2010) Assessing permethrin resistance in the stable fly (Diptera: Muscidae) in Florida by using laboratory selections and field evaluations. *Journal of Economic Entomology*, **103**, 2258–2263.

Pitzer, J.B., Kaufman, P.E., Geden, C.J. and Hogsette, J.A. (2011) The ability of selected pupal parasitoids (Hymenoptera: Pteromalidae) to locate stable fly hosts in a soiled equine bedding substrate. *Environmental Entomology*, **40**, 88–93.

Pouokam, G.B., Album, W.L., Ndikontar, A.S. and Sidatt, M.E.H. (2017) A pilot study in cameroon to understand safe uses of pesticides in agriculture, risk factors for farmers' exposure and management of accidental cases. *Toxics*, **5**, 1–15.

Priestley, C.M., Williamson, E.M., Wafford, K.A. and Sattelle, D.B. (2003) Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human GABA A receptors and a homo-oligomeric GABA receptor from Drosophila melanogaster. *British Journal of Pharmacology*, **140**, 1363–1372.

Rajkumar, V., Gunasekaran, C., Christy, I.K., Dharmaraj, J., Chinnaraj, P. and Paul, C.A. (2019) Toxicity, antifeedant and biochemical efficacy of *Mentha piperita* L. essential oil and their major constituents against stored grain pest. *Pesticide Biochemistry and Physiology*, **156**, 138-144.

Rivaroli, D.C., Guerrero, A., Valero, M.V., Zawadzki, F., Eiras, C.E., del Mar Campo, M., Sañudo, C.,

Jorge, A.M. and do Prado, I.N. (2016) Effect of essential oils on meat and fat qualities of crossbred young bulls finished in feedlots. *Meat Science*, **121**, 278-284.

Rowshan, V., Bahmanzadegan, A. and Saharkhiz, M.J. (2013) Influence of storage conditions on the essential oil composition of *Thymus daenensis* Celak. *Industrial Crops and Products*, **49**, pp.97-101.

Russell, M. (1999) 'Toxicology of tea tree oil', *In* I. Southwell and R. Lowe (ed.) *Tea tree: the* genus *Melaleuca*. Amsterdam: Harwood Academic Publishers, 191-201.

Salem, A., Bouhsira, E., Liénard, E., Melou, A.B., Jacquiet, P. and Franc, M. (2012) Susceptibility of two European strains of *Stomoxys calcitrans* (L.) to cypermethrin, deltamethrin, fenvalerate, λ-cyhalothrin, permethrin and phoxim. *International Journal of Applied Research in Veterinary Medicine*, **10**, 249–257.

Sands, B., Ellse, L. and Wall, R. (2016) Residual and ovicidal efficacy of essential oil-based formulations in vitro against the donkey chewing louse *Bovicola ocellatus*. *Medical and Veterinary Entomology*, **30**, 78–84.

Sands, B., Mgidiswa, N., Nyamukondiwa, C. and Wall, R. (2018) Environmental consequences of deltamethrin residues in cattle feces in an African agricultural landscape. *Ecology and Evolution*, **8**, 2938–2946.

Savelev, S., Okello, E., Perry, N.S.L., Wilkins, R.M. and Perry, E.K. (2003) Synergistic and antagonistic interactions of anticholinesterase terpenoids in *Salvia lavandulaefolia* essential oil. *Pharmacology Biochemistry and Behavior*, 75, 661–668.

Schmidt, E., Bail, S., Buchbauer, G., Stoilova, I., Atanasova, T., Stoyanova, A., Krastanova, A. and Jirovetz, L. (2009) Chemical composition, olfactory evaluation and antioxidant effects of essential oil from *Mentha x piperita*. *Natural Product Communications*, **4**, 1107–1112.

Schowalter, T. D. and Klowden M. J. (1979) Blood meal size of the stable fly, *Stomoxys calcitrans*, measured by the HiCN method. *Mosquito New*, **39**, 110-112.

Schwinghammer, K.A., Knapp, F.W. and Boling, J.A. (1987) Physiological and nutritional response of beef steers to combined infestations of horn fly and stable fly (Diptera: Muscidae). *Journal of Economic Entomology*, **80**, 1294–1298.

Sfara, V., Zerba, E.N. and Alzogaray, R.A. 2009. Fumigant insecticidal activity and repellent effect of five essential oils and seven monoterpenes on first-instar nymphs of *Rhodnius prolixus. Journal of Medical Entomology*, **46**, 511–515.

Shaw, A.O. and Atkeson, F.W. (1943) Effect of spraying cows with repellent type sprays as measured by milk production. *Journal of Dairy Science*, **26**, 179–187.

Shetta, A., Kegere, J. and Mamdouh, W. (2019) Comparative study of encapsulated peppermint and

green tea essential oils in chitosan nanoparticles: Encapsulation, thermal stability, in-vitro release, antioxidant and antibacterial activities. *International Journal of Biological Macromolecules*, **126**, 731–742.

Showler, A.T. (2017) Botanically based repellent and insecticidal effects against horn flies and stable flies (Diptera: Muscidae). *Journal of Integrated Pest Management,* **8**, 1–11.

Showler, A.T. and Osbrink, W.L.A. (2015) Stable fly*, Stomoxys calcitrans* (L.), dispersal and governing factors. *International Journal of Insect Science*, **7**, 19–25.

Sienkiewicz, M., Głowacka, A., Kowalczyk, E., Wiktorowska-Owczarek, A., Jóźwiak-Bębenista, M. and Łysakowska, M. (2014) The biological activities of cinnamon, geranium and lavender essential oils. *Molecules*, **19**, 20929-20940.

Sikkema, J., de Bont, J.A. and Poolman, B. (1995) Mechanisms of membrane toxicity of hydrocarbons. *Microbiological Reviews*, **59**, 201-222.

Silvestre, A.J.D., Cavaleiro, J.A.S., Delmond, B., Filliatre, C. and Bourgeois, G. (1997) Analysis of the variation of the essential oil composition of *Eucalyptus globulus* Labill. from Portugal using multivariate statistical analysis. *Industrial Crops and Products*, **6**, 27–33.

Skovgård, H. (2004) Sustained releases of the pupal parasitoid *Spalangia cameroni* (Hymenoptera: Pteromalidae) for control of house flies, *Musca domestica* and stable flies *Stomoxys calcitrans* (Diptera: Muscidae) on dairy farms in Denmark. *Biological Control,* **30**, 288–297.

Skovgård, H. and Nachman, G. (2012) Population dynamics of stable flies *Stomoxys calcitrans* (Diptera: Muscidae) at an organic dairy farm in Denmark based on mark-recapture with destructive sub-sampling. *Environmental Entomology*, **41**, 20–29.

Suwannayod, S., Sukontason, K., Pitasawat, B., Junkum, A., Limspatham, K., Jones, M. and Somboon, P. (2019) Synergistic toxicity of plant essential oils combined with pyrethroid insecticides against blow flies and the house fly. *Insects,* **10**, 1–16.

Swist, S.L., Wilkerson, M.J., Wyatt, C.R., Broce, A.B. and Kanost, M.R. (2002) Modulation of bovine lymphocyte response by salivary gland extracts of the stable fly, *Stomoxys calcitrans* (Diptera: Muscidae). *Journal of Medical Entomology*, **39**, 900–907.

Talbert, R. and Wall, R. (2012) Toxicity of essential and non-essential oils against the chewing louse, *Bovicola (Werneckiella) ocellatus*. *Research in Veterinary Science*, **93**, 831–835.

Tawatsin, A., Thavara, U., Chansang, U., Chavalittumrong, P. and Boonruad, B. (2006) Field evalutation of DEET, Repel Care, and three plant based essential oil repellents against mosquitoes, black flies (Diptera: Simulidae) and land leeches (Arhynchobdellida) in Thailand. *Journal of American Mosquito Control Association*, **22**, 306–313.

Tawatsin, A., Wratten, S.D., Scott, R.R., Thavara, U., and Techadamrongsin, Y. (2001) Repellency of

volatile oils from plants against three mosquito vectors. *Journal of Vector Ecology*, **26,** 76–82.

Taylor, D.B. and Berkebile, D. (2006) Comparative efficiency of six stable fly (Diptera: Muscidae) traps. *Journal of Economic Entomology*, **99**, 1415–1419.

Taylor, D.B., Friesen, K. and Zhu, J. (2017) Precipitation and temperature effects on stable fly (Diptera: Muscidae) population dynamics. *Environmental Entomology*, **46**, 434–439.

Taylor, D.B., Moon, R.D. and Mark, D.R. (2012) Economic Impact of Stable Flies (Diptera: Muscidae) on Dairy and Beef Cattle Production. *Journal of Medical Entomology*, **49**, 198–209.

Todd, D.H. (1964) The biting fly *Stomoxys calcitrans* (L.) in dairy herds in New Zealand. *New Zealand Journal of Agricultural Research*, **7**, 60–79.

Toledo, P.F.S., Ferreira, T.P., Bastos, I.M.A.S., Rezende, S.M., Viteri Jumbo, L.O., Didonet, J., Andrade, B.S., Melo, T.S., Smagghe, G., Oliveira, E.E. and Aguiar, R.W.S. (2019) Essential oil from Negramina (*Siparuna guianensis*) plants controls aphids without impairing survival and predatory abilities of non-target ladybeetles. *Environmental Pollution*, **255**, 1–12.

Tong, F. and Coats, J.R. (2010) Effects of monoterpenoid insecticides on [3H]-TBOB binding in house fly GABA receptor and 36Cl- uptake in American cockroach ventral nerve cord. *Pesticide Biochemistry and Physiology*, **98**, 317–324.

Traversa, D., Otranto, D., Iorio, R., Carluccio, A., Contri, A., Paoletti, B., Bartolini, R. and Giangaspero, A. (2008) Identification of the intermediate hosts of *Habronema microstoma* and *Habronema muscae* under field conditions. *Medical and Veterinary Entomology*, **22**, 283–287.

Turek, C. and Stintzing, F.C. (2013) Stability of essential oils: A review. *Comprehensive Reviews in Food Science and Food Safety,* **12**, 40–53.

Turell, M.J., Dohm, D.J., Geden, C.J., Hogsette, J.A. and Linthicum, K.J. (2010) Potential for stable flies and house flies (Diptera: Muscidae) to transmit rift valley fever virus. *Journal of the American Mosquito Control Association*, **26,** 45-448.

Turell, M.J., Dohm, D.J., Geden, C.J., Hogsette, J.A. and Linthicum, K.J. (2010) Potential for stable flies and house flies (Diptera: Muscidae) to transmit rift valley fever virus. *Journal of the American Mosquito Control Association*, **26**, 445–448.

Uddin, M.H., Shahjahan, M., Amin, A.R., Haque, M.M., Islam, M.A. and Azim, M.E. (2016) Impacts of organophosphate pesticide, sumithion on water quality and benthic invertebrates in aquaculture ponds. *Aquaculture Reports*, **3**, 88-92.

Urban, J.E. and Broce, A. (1998) Flies and their bacterial loads in greyhound dog kennels in Kansas. *Current Microbiology*, **36**, 164–170.

Vetere, A., Bertocchi, M., Pelizzone, I., Moggia, E., Travaglino, C., Della Grotta, M., Casali, S., Gerosa, S., Strada, L., Filia, K., Casalini, J., Parmigiani, E. and Di Ianni, F. (2020) Acute tea tree oil

intoxication in a pet cockatiel (*Nymphicus hollandicus*): A case report. *BioMed Central Veterinary Research*, **16**, 1–5.

Viljoen, A.M., Subramoney, S., Vuuren, S.F.V., Başer, K.H.C. and Demirci, B. (2005) The composition, geographical variation and antimicrobial activity of *Lippia javanica* (Verbenaceae) leaf essential oils. *Journal of Ethnopharmacology*, **96**, 271–277.

Vitela-Mendoza, I., Cruz-Vázquez, C., Solano-Vergara, J. and Orihuela-Trujillo, A. (2016) Short communication: Relationship between serum cortisol concentration and defensive behavioral responses of dairy cows exposed to natural infestation by stable fly, *Stomoxys calcitrans*. *Journal of Dairy Science,* **99**, 9912–9916.

Wang, Q., Reddy, V.A., Panicker, D., Mao, H.Z., Kumar, N., Rajan, C., Venkatesh, P.N., Chua, N.H. and Sarojam, R. (2016) Metabolic engineering of terpene biosynthesis in plants using a trichome-specific transcription factor Ms YABBY 5 from spearmint (*Mentha spicata*). *Plant Biotechnology Journal*, **14**, 1619-1632.

Wanzala, W., Hassanali, A., Mukabana, W.R. and Takken, W. (2014) Repellent activities of essential oils of some plants used traditionally to control the brown ear tick, *Rhipicephalus appendiculatus*. *Journal of Parasitology Research*, **2014**, 1-10.

Wanzala, W., Takken, W., Mukabana, W.R., Pala, A.O. and Hassanali, A. (2012) Ethnoknowledge of Bukusu community on livestock tick prevention and control in Bungoma district, western Kenya. *Journal of Ethnopharmacology*, **140**, 298–324.

Weaving, H., Sands, B. and Wall, R. (2020) Reproductive sublethal effects of macrocyclic lactones and synthetic pyrethroids on the dung beetle *Onthophagus similis*. *Bulletin of Entomological Research*, **110,** 195-200.

Weinzierl, R.A. and Jones, C.J. (1998) Releases of *Spalangia Nigroaenea* and *Muscidifurax Zaraptor* (Hymenoptera: Pteromalidae) increase rates of parasitism and total mortality of stable fly and house fly (Diptera: Muscidae) pupae in Illinois cattle feedlots. *Journal of Economic Entomology*, **91**, 1114–1121.

Wieman, G. A., Campbell, J. B., Deshazer, J. A., & Berry, I. L. (1992). Effects of stable flies (Diptera: Muscidae) and heat stress on weight gain and feed efficiency of feeder Cattle. *Journal of Economic Entomology,* **85**, 1835–1842.

Williams, D.F. (1973) Sticky traps for sampling population of *Stomoxys calcitrans. Journal of Economic Entomology,* **66**, 1279–1280.

World Health Organisation (2009) Guidelines for Efficacy Testing of Mosquito Repellents for Human Skin. Control of Neglected Tropical Diseases WHO Pesticide Evaluation Scheme. *World Health Organisation*, 4-5.

World Health Organisation (2018) Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes. Global Malaria Programme. *World Health Organisation*, 9-19.

Yadav, E., Kumar, S., Mahant, S., Khatkar, S. and Rao, R. (2017) Tea tree oil: a promising essential oil. Journal of Essential Oil Research, **29**, 201–213.

Yaghoobi-Ershadi, M.R., Akhavan, A.A., Jahanifard, E., Vatandoost, H., Amin, G., Moosavi, L., Zahraei Ramazani, A.R., Abdoli, H. and Arandian, M.H. (2006) Repellency effect of Myrtle essential oil and DEET against *Phlebotomus papatasi*, under laboratory conditions. *Iranian Journal of Public Health*, **35**, 7–13.

Yeom, H.J., Jung, C.S., Kang, J., Kim, J., Lee, J.H., Kim, D.S., Kim, H.S., Park, P.S., Kang, K.S. and Park, I.K. (2015) Insecticidal and acetylcholine esterase inhibition activity of asteraceae plant essential oils and their constituents against adults of the German cockroach (*Blattella germanica*). *Journal of Agricultural and Food Chemistry*, **63**, 2241–2248.

Yeruham, I. and Braverman, Y. (1995) Skin lesions in dogs, horses and calves caused by the stable fly *Stomoxys calcitrans* (L.) (Diptera: Muscidae). *Revue d'élevage et de Médecine Vétérinaire des Pays Tropicaux*, **48**, 347–349.

Zeil, J. (1983) Sexual dimorphism in the visual system of flies: The free flight behaviour of male *Bibionidae* (Diptera). *Journal of Comparative Physiology*, **150**, 395–412.

Zhu, J.J., Berkebile, D.R., Dunlap, C.., Zhang, A., Boxler, D., Tangtrakulwanich, K., Behle, R.., Baxendale, F. and Brewer, G. (2012) Nepetalactones from essential oil of Nepeta cataria represent a stable fly feeding and oviposition repellent. *Medical and Veterinary Entomology,* **26**, 131–138.

Zhu, J.J., Dunlap, C.A., Behle, R.W., Berkebile, D.R. and Wienhold, B. (2010) Repellency of a wax-based catnip-oil formulation against stable flies. *Journal of agricultural and food chemistry*, **58**, 12320-12326.

Zhu, J.J., Li, A.Y., Pritchard, S., Tangtrakulwanich, K., Baxendale, F. and Brewer, G. (2011) Contact and fumigant toxicity of a botanical-based feeding deterrent of the stable fly, *Stomoxys calcitrans* (Diptera: Muscidae). *Journal of Agricultural and Food Chemistry*, **59**, 10394–10400.

Zhu, J.J., Wienhold, B.J., Wehrle, J., Davis, D., Chen, H., Taylor, D., Friesen, K. and Zurek, L. (2014) Efficacy and longevity of newly developed catnip oil microcapsules against stable fly oviposition and larval growth. *Medical and Veterinary Entomology,* **28,** 222–227.

Zhu, J.J., Zeng, X.P., Berkebile, D., Du, H.J., Tong, Y. and Qian, K. (2009) Efficacy and safety of catnip (*Nepeta cataria*) as a novel filth fly repellent. *Medical and Veterinary Entomology,* **23**, 209–216.

Zumpt, F. (1973) *The stomoxyine biting flies of the world*. Stuttgart: G. Fischer.

Appendix

Appendix I. An enumeration of essential oils which have been investigated for their repellent or insecticidal properties against biting flies of veterinary importance.

Plant Family	Plant Species (Common name)	Fly Species (Common name)	Bioassay	Results	Reference
Amaryllidaceae	*Allium cepa* (Onion)	Variety	IV	2.9 mL/kg of buffalo body weight deterred flies for 6 days.	Khater *et al*., 2009
	Allium sativum (Garlic)	*Cephalopina titillator* (Camel nasal botfly)	LIB	LD_{50} was 0.44% (v/v).	Khater, 2014.
		Calliphora vomitoria (blue bottle blowfly)	TA	LD_{50} was 22% (v/v).	Bedini *et al.,* 2020
Apiaceae	*Coriandrum sativum* (Coriander)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 12 minutes.	Hieu *et al.,* 2010
	Levisticum officinale (Lovage)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 3.36 hours.	Hieu *et al.,* 2010
	Pimpinella anisum (Anise)	*Lucilia sericata* (Common green bottle fly)	IB	LD_{50} for larvae was 2.74% (v/v).	Khater *et al.,* 2011
Araceae	*Homalomena aromatica* Schott	*Simulium* spp. (Blackflies)	SB	5% (v/v) repelled flies for 2.13 hours.	Hazarika *et al.,* 2012
Asteraceae	*Ageratum conzoides* (Billygoat-weed)	*Simulium* spp. (Blackflies)	SB	5% (v/v) repelled flies for 2.85 hours.	Hazarika *et al.,* 2012
	Artemesia vulgaris (Armoise)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 of repelled flies for 20 minutes.	Hieu *et al.,* 2010
	Baccharis dracunculifolia	*Cochliomyia macellaria* (Secondary screwworm)	FPB	LD_{50} for larvae was 2.63µL/cm^2.	Chaaban *et al.,* 2018

	Espeletia shultzii	*Lutzomyia migonei*	SB	0.416 µL/cm^2 repelled flies for 32 minutes.	Nieves *et al.,* 2010
	Lactuca sativa (Lettuce)	*Lucilia sericata* (Common green bottle fly)	IB	LD_{50} was 0.57% (v/v).	Khater *et al.,* 2011
	Matricaria chamomilla (Chamomile)	Variety	IV	3.4 mL/kg of buffalo body weight deterred flies from buffaloes for 6 days.	Khater et al., 2009
		Lucilia sericata (Common green bottle fly)	IB	LD_{50} was 0.85% (v/v).	Khater *et al.,* 2011
	Monticalia greenmaniana	*Lutzomyia migonei*	FPB	0.1 mg/mL caused 100% mortality 1 hour post exposure	Cárdenas *et al.,* 2012
	Monticalia imbricatifolia	*Lutzomyia migonei*	SB	0.416 µL/cm^2 repelled flies for 1.45 hours.	Nieves *et al.,* 2010
	Pseudognaphalium caeruleocanum	*Lutzomyia migonei*	SB	0.416 µL/cm^2 repelled flies for 5 hours.	Nieves *et al.,* 2010
Cucurbitaceae	*Cucurbita maxima* (Pumpkin)	*Cephalopina titillator* (Camel nasal botfly)	LIB	LD_{50} was 0.20% (v/v).	Khater, 2014.
Geraniaceae	*Pelargonium graveolens* (Geranium)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 1.11 hours.	Hieu *et al.,* 2010
		Variety	IV	5% (v/v) significantly reduced the abundance of flies on heifers for 3 hours.	Lachance and Grange 2014
Fabaceae	*Lupinus luteus* (Yellow lupin)	*Cephalopina titillator* (Camel nasal botfly)	LIB	LD_{50} was 0.42% (v/v).	Khater, 2014.

Lamiaceae	*Clinopodium nubigenum* (Kunth)	*Lucilia sericata* (Common green bottle fly)	FPB	LD_{50} for eggs and adults was 0.07 µL/cm^2 and 0.278 µL/cm^2, respectively.	Bedini *et al.,* 2019
	Hyptis suaveolens (Pignut)	*Lutzomyia migonei*	SB	No repellent effect.	Nieves *et al.,* 2010
	Lavandula angustifolia (English Lavender)	*Lucilia sericata* (Common green bottle fly)	BB	LD_{50} was 0.063% (v/v), 5 minutes post exposure.	Khater and Geden 2018
		Lucilia sericata (Common green bottle fly)	FPB	LD_{50} for eggs and adults was 0.48 µL/cm^2 and 0.393 µL/ cm^2, respectively.	Bedini *et al.,* 2019
		Variety	IV	5% (v/v) significantly reduced the abundance of flies on heifers for 3 hours.	Lachance and Grange 2014
		Culicoides obsoletus	DCB	1 µg/µL repelled 93.7% of flies for 4 minutes.	González *et al.,* 2014
		Stomoxys calcitrans (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 29 minutes.	Hieu *et al.,* 2010
	Lavandula dentata (French Lavender)	*Chrysomya albiceps Wiedemann*	FPB	LD_{50} for adults was 5.14% (lw/v).	Cossetin *et al.,* 2018
	Melissa officinalis (Lemon balm)	*Culicoides obsoletus*	DCB	1 µg/µL repelled 88.4% of flies for 4 minutes.	González *et al.,* 2014
	Mentha piperita (Peppermint)	*Cephalopina titillator* (Camel nasal botfly)	LIB	LD_{50} was 0.47% (v/v).	Khater, 2014.
		Variety	*In vivo*	3.6 mL/kg of buffalo body weight deterred flies for 6 days.	Khater et al., 2009
		Variety	IV	5% (v/v) significantly reduced the abundance of flies on heifers for 3 hours.	Lachance and Grange 2014
	Plectranthus amboinicus	*Lutzomyia migonei*	SB	0.416 µL/cm^2 repelled flies for 4.18 hours	Nieves *et al.,* 2010

Nepeta cataria (Catnip)	*Stomoxys calcitrans* (Stable fly)	NCB	66 µg/µL repelled 97% of flies for 4 hours.	Zhu *et al.,* 2009.
	Stomoxys calcitrans (Stable fly)	TA FT	50 µg/fly caused 100% mortality when topically applied and the fumigant LD_{50} was 10.7 mg/cm^3.	Zhu *et al.,* 2011
	Stomoxys calcitrans (Stable fly)	NCB IV	67 µg/µL repelled 96% from feeding for 4 hours. *In vivo*, 15% (v/v) EO repelled flies for 6 hours.	Zhu *et al.,* 2012
	Haematobia irritans (Horn fly)	NCB	0.67 µg/µL in hexane, repelled 85% of flies for 4 hours.	Zhu *et al.,* 2015
Ocimum basilicum (Basil)	Variety	IV	5% (v/v) significantly reduced the abundance of flies on heifers for 3 hours.	Lachance and Grange 2014
Ocimum gratissimum	*Lucilia cuprina* (Australian sheep blowfly) *Chrysomya megacephala* (Oriental latrine fly) *Chrysomya rufifacies* (Hairy maggot blowfly)	TA	LD_{50} foradults was 110, 166 and 68.5 µg/fly for the three species, respectively.	Suwannayod *et al.,* 2019
Ocimum sanctum var. cubensis (Holy basil)	*Chrysomya putoria* (African latrine blowfly)	TA	LD_{50} for larvae was 7.47 mg/mL.	Chil-Núñez *et al.,* 2018.
Origanum majorana (Marjoram)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 7 minutes.	Hieu *et al.,* 2010
Origanum vulgare (Oregano)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 1.15 hours.	Hieu *et al.,* 2010
Pogostemon cablin (Blanco) (Patchouli)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 3.67 hours.	Hieu *et al.,* 2010
Pogostemon heyneanus	*Simulium* spp. (Blackflies)	IV SB	5% (v/v) provided protection for 1.11 hours.	Hazarika *et al.,* 2012
Rosmarinus officinalis (Rosemary)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 13 minutes.	Hieu *et al.,* 2010

		Lucilia sericata (Common green bottle fly)	IB	LD_{50} was 6.77% (v/v).	Khater *et al.,* 2011
		Culicoides obsoletus	DCB	1 µg/µL repelled 70% of flies for 4 minutes.	González *et al.,* 2014
		Calliphora vomitoria (blue bottle blowfly)	TA	LD_{50} was 55% (v/v).	Bedini *et al.,* 2020
	Salvia officinalis (common sage)	*Calliphora vomitoria* (blue bottle blowfly)	TA	LD_{50} was 99% (v/v).	Bedini *et al.,* 2020
	Salvia sclerea (Sage)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 30 minutes.	Hieu *et al.,* 2010
	Satureja monata (Savory)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 did not repel flies.	Hieu *et al.,* 2010
	Thymus vulgaris (Thyme)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 2.12 hours.	Hieu *et al.,* 2010
	Vitex negundo	*Simulium* spp. (Blackflies)	IV SB	5% (v/v) EO provided protection for 2.68 hours	Hazarika *et al.,* 2012
Lauraceae	*Cinnamomum camphora* (Camphor)	Variety	IV	1.4 mL/kg of buffalo body weight deterred flies for 6 days	Khater et al., 2009
	Cinnamomum verum (True cinnamon tree)	*Lucilia sericata* (Common green bottle fly)	BB	LD_{50} for adults was 0.079% (v/v), 5 minutes post exposure.	Khater and Geden 2018
		Lutzomyia migonei	SB	100% EO provided protection for 4.2 hours	Nieves *et al.,* 2010
Meliaceae	*Carapa guianensis* (Andiroba)	*Haematobia irritans (L.)* (Horn fly)	TA IV	1% (v/v) caused 100% mortality 4 hours post treatment. 5% (v/v) significantly reduced flies on cattle for 6 hours.	Klauck *et al.,* 2014

		Haematobia irritans (L.) (Horn fly)	FR	5% (v/v) repelled flies for 3 hours.	Klauck *et al.,* 2015
Myrtaceae	*Corymbia citriodora* (Lemon-scented gum)	*Lutzomyia longipalpis*	BB	10% (v/v) achieved 88.13% mortality 24 hours post treatment.	Maciel *et al.,* 2010
		Culicoides obsoletus	DCB	1 µg/µL repelled 90.5% of flies.	González *et al.,* 2014
	Eucalyptus globules (Eucalyptus)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 8 minutes.	Hieu *et al.,* 2010
		Lutzomyia longipalpis	BB	10% (v/v) achieved 95.50% mortality 24 hours post treatment.	Maciel *et al.,* 2010
	Eucalyptus staigeriana (Lemon-scented ironbark)	*Lutzomyia longipalpis*	BB	5% (v/v) achieved 99.62% mortality 24 hours post treatment.	Maciel *et al.,* 2010
	Eugenia caryophyllata (Clove)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 3.5 hours.	Hieu *et al.,* 2010
	Melaleuca alternifolia (Tea tree)	*Haematobia irritans (L.)* (Horn fly)	TA IV	1% (v/v) EO caused 100% mortality 4 hours post treatment. 5% (v/v) EO significantly reduced flies on cattle for 24 hours.	Klauck *et al.,* 2014
		Lucilia cuprina (Australian sheep blowfly)	DCB FPB IB	1% (v/v) formulation caused 100% ovicidal and larvicidal mortality. 3% (v/v) solution prevented oviposition of gravid females for 6 weeks.	Callander and James 2012.
		Haematobia irritans (L.) (Horn fly)	FR	5% (v/v) repelled flies for 2 hours.	Klauck *et al.,* 2015
		Variety	IV	5% (v/v) significantly reduced the abundance of flies on heifers for 8 hours.	Lachance and Grange 2014

	Myrtus communis (Myrtle)	*Phlebotomus papatasi*	NCB	ED_{50} to repel flies was for 5 minutes was 0.114 mg/cm^2	Yaghoobi-Ershadi *et al.,* 2006
	Pimenta racemose (West Indian bay tree)	*Lutzomyia migonei*	SB	No repellent effect	Nieves *et al.,* 2010
	Psidium guajava (Common guava)	*Simulium* spp. (Blackflies)	IV SB	10% (w/w) EO formulation provided 100% protection for 9 hours.	Tawatsin *et al.,* 2006
Oleaceae	*Jasminum grandiflorum* (Jasmine)	*Culicoides obsoletus*	DCB	1 µg/µL repelled 93.9% of flies.	González *et al.,* 2014
Pinaceae	*Pinus sylvestris* (Pine)	Variety	IV	5% (v/v) EO caused significantly lower number of flies on individual heifers 2 hours post treatment.	Lachance and Grange 2014
Piperaceae	*Piper gaudichaudianum* (Piper)	*Lucilia cuprina* (Australian sheep blowfly)	FPB	LD_{50} against larvae was 2.19 µL/cm^2, 48 hours post exposure.	Chaaban *et al.,* 2018
	Piper marginatum (Marigold pepper)	*Lutzomyia migonei*	SB	No repellent effect.	Nieves *et al.,* 2010
Poaceae	*Chrysopogon zizanioides* (Vetiver)	*Lucilia sericata* (Common green bottle fly)	BB	LD_{50} for adults was 0.082% (v/v), 5 minutes post exposure.	Khater and Geden 2018
	Cymbopogon citratus (Citronella)	*Stomoxys calcitrans* (Stable fly)	DCB	0.1 mg/µL EO repelled flies from feeding for 10 minutes.	Baldacchino *et al.,* 2013
		Variety	IV	5% (v/v) EO significantly reduced the abundance of flies on heifers for 3 hours.	Lachance and Grange 2014
		Culicoides obsoletus	DCB	1 µg/µL repelled 72.7% of flies.	González *et al.,* 2014
		Stomoxys calcitrans (Stable fly)	*IV*	6% (v/v) EO formulation significantly reduced fly annoyance behaviours in horses for 2 hours.	Mottet *et al.,* 2018

	Cymbopogon nardus (Citronella)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 16 minutes.	Hieu *et al.,* 2010
		Haematobia irritans (L.) (Horn fly)	FR	5% (v/v) EO diluted with triton-water solution repelled flies for 2 hours.	Klauck *et al.,* 2015
Rutaceae	*Amyris balsamifera* (West Indian sandalwood)	*Stomoxys calcitrans* (Stable fly)	NCB	67 µg/µL repelled 55% of flies from feeding for 4 hours.	Zhu *et al.,* 2012
	Citrus aurantifolia Swingle	*Simulium* spp. (Blackflies)	IV SB	5% (v/v) EO provided protection for 52 minutes.	Hazarika *et al.,* 2012
	Citrus bergamia (Risso) (Bergamot)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 37 minutes.	Hieu *et al.,* 2010
	Zanthoxylum armatum (Xanthoxylum)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 35 minutes.	Hieu *et al.,* 2010
		Stomoxys calcitrans (Stable fly)	SB	0.20 mg/cm^2 repelled 91% of flies for 30 minutes.	Hieu *et al.,* 2010a
		Stomoxys calcitrans (Stable fly)	DCB	0.06 mg/µL repelled 86% of flies for 15 minutes.	Hieu *et al.,* 2014
	Zanthoxylum piperitum (Japanese pepper)	*Stomoxys calcitrans* (Stable fly)	DCB	0.06 mg/µL repelled 87% of flies for 15 minutes.	Hieu *et al.,* 2014
		Stomoxys calcitrans (Stable fly)	SB	0.4 mg/cm^2 repelled 72% of flies for 1.5 hours.	Hieu *et al.,* 2010a
Santalaceae	*Santalum album* (Sandalwood)	*Stomoxys calcitrans* (Stable fly)	SB	0.5 mg/cm^2 repelled flies for 16 minutes.	Hieu *et al.,* 2010
		Stomoxys calcitrans (Stable fly)	NCB	67 µg/µL repelled 70% of flies from feeding for 4 hours.	Zhu *et al.,* 2012
Zingiberaceae	*Boesenbergia rotunda* (Fingerroot)	*Simulium* spp. (Blackflies)	IV SB	10% (w/w) provided 100% protection for 9 hours.	Tawatsin *et al.,* 2006

		Lucilia cuprina (Australian sheep blowfly) *Chrysomya megacephala* (Oriental latrine fly) *Chrysomya rufifacies* (Hairy maggot blowfly)	TA	LD_{50} against adults was 207, 250 and 104 µg/fly for the three species, respectively.	Suwannayod *et al.,* 2019
	Curcuma longa (Tumeric)	*Simulium* spp. (Blackflies)	IV SB	10% (w/w) EO formulation provided 100% protection for 9 hours.	Tawatsin *et al.,* 2006
		Cochliomyia macellaria (Secondary screw-worm)	FPB	LD_{50} against larvae was 0.84 µL/cm^2 48 hours post exposure.	Chaaban *et al.,* 2019b
		Lucilia cuprina (Australian sheep blowfly)	FPB	LD_{50} against larvae was 1.34 µL/cm^2 6 hours post exposure.	Chaaban *et al.,* 2019a
		Lucilia cuprina (Australian sheep blowfly) *Chrysomya megacephala* (Oriental latrine fly) *Chrysomya rufifacies* (Hairy maggot blowfly)	TA	LD_{50} against adults was 94.52, 129.73 and 59.83 µg/fly for the three species, respectively.	Suwannayod *et al.,* 2019

Bioassays used for assessment of essential oils: LIB, Larval Immersion Bioassay; TA, Topical Application; IB, Ingestion Bioassay; BB, Bottle Bioassay; FPB, Filter Paper Bioassay; FT, Fumigant Toxicity; DCB, Dual Choice Bioassay; NCB, No Choice Bioassay; SB, Skin Bioassay; IV, *In Vivo.*